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Tissue-specific reactivation of gene expression at an imprinted locus

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Abstract

Genomic imprinting is the phenomenon where the expression pattern of an allele at a locus differs depending on the allele's parent of origin. In most cases, one of the two alleles is transcriptionally silent. Recent empirical work has shown some genes to be imprinted in a tissue-specific manner, where the silenced allele becomes reactivated in particular cell lineages during development. Here I describe an evolutionary model of tissue-specific transcriptional reactivation. The model describes the relationships among various inclusive fitness functions and phenotypic effects necessary for natural selection to favor the epigenetic reprogramming required for this sort of reactivation, and makes predictions regarding the nature and magnitude of phenotypic and fitness consequences of mutations in particular somatic tissues. In particular, if an imprinted gene is reactivated in one of two tissues that interact in producing a particular phenotype, expression of the gene in those two tissues is expected to have opposite phenotypic effects. The model predicts that in some cases, mutations affecting the silenced allele at an imprinted locus may be phenotypically more severe than those affecting the expressed allele. These predictions are contrasted with those of an alternative explanation for reactivation: protection against deleterious recessive somatic mutations. The inclusive-fitness model of reactivation indicates that the intragenomic conflicts present in the parental germ lines and developing embryo persist though adult life, and can have complex effects on phenotypes and patterns of gene expression in somatic tissues.

Keywords: Genomic imprinting; Epigenetics; Intragenomic conflict; Gene expression

1. Introduction

At an imprinted locus an allele's pattern of expression depends on its parent of origin (for recent reviews see, e.g. Reik and Walter, 2001a; Sleutels and Barlow, 2002; Murphy and Jirtle, 2003). In the simplest cases, transcription is silenced from one of the two alleles, although in some cases imprinting can be isoform-specific, with maternal-specific, paternal-specific, and biallelic expression of different splicing variants from the same locus. Imprinted gene expression results from reversible epigenetic modifications that are established differentially in the male and female gametes each generation and propagated in an allele-specific manner in the cell divisions following fertilization (Li, 2002; Jaenisch and Bird, 2003; Rand and Cedar, 2003). These epigenetic modifications include direct modification of the DNA by cytosine methylation as well as modifications to associated proteins, such as methylation and deacetylation of histones.

The kinship theory of imprinting attributes the evolution of imprinted gene expression to a conflict of interests between the maternally and paternally derived alleles at a locus (Haig, 2002; Wilkins and Haig, 2003a). That is, the level of gene expression that maximizes the inclusive fitness of an allele can differ depending on whether that allele was present in a male or a female in the previous generation. The origin of the inclusive-fitness asymmetry driving the evolution of imprinting has been most thoroughly characterized in the context of mammalian reproduction, where genes expressed in the fetus can influence the distribution of maternal resources. Due to the possibility of multiple paternity, alleles present in the fetus benefit from placing a greater demand on maternal resources when they are

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paternally derived than when they are maternally derived. At imprinted loci, where alleles can evolve two separate expression patterns conditional on parent of origin, genes that increase the demand placed on the mother are transcriptionally silent from the maternally derived allele, whereas those that reduce demand are paternally silenced (Mochizuki et al., 1996; Haig, 1997; Wilkins and Haig, 2001; Mills and Moore, 2004).

While most imprinted genes modulate fetal growth, many of these genes are also associated with phenotypes that emerge later in development (Tycko and Morrison, 2002). Furthermore, some of these genes show tissue-specific patterns of expression, with biallelic expression in some cell types and monoallelic expression in others. The logic of the kinship theory applies to any trait with asymmetric consequences for matrilineal and patrilineal kin (individuals to whom one is related through one's mother or father, respectively), and provides a framework in which to interpret these later phenotypes and complex expression patterns. In practice, however, it is often difficult to study the phenotypic effects of tissue-specific changes in gene expression, much less to understand how these effects alter the matrilineal and patrilineal inclusive fitness.

Some postnatal phenotypes associated with imprinted genes can be understood by direct analogy with prenatal growth effects. For instance, some imprinted genes expressed in offspring are associated with weaning and suckling behaviors (Curley et al., 2004; Plagge et al., 2004; Isles and Holland, 2005), and represent simply a different mechanism for influencing the distribution of maternal resources (Haig and Wharton, 2003). In other instances, imprinted genes are expressed in adult neural tissues (Isles and Wilkinson, 2000), where they can potentially influence behaviors whose fitness effects might be mediated through complex social interactions.

One adult behavior affected by imprinted genes is maternal care for offspring. Knockouts of two paternally expressed loci, *Peg1/Mest* (Lefebvre et al., 1998) and *Peg3* (Li et al., 1999), result in defects in placentophagy, pup retrieval, and nest building. These results suggest that a mother's paternally derived allele favor higher investment in her current litter than does her maternally derived allele, despite the fact that each of these alleles is equally likely to be transmitted to each of her offspring. Wilkins and Haig (2003b) suggested a model in which patterns of inbreeding change over the course of a female's life. An intragenomic conflict arises within the mother over the distribution of resources between her early and late litters, with her paternally derived allele favoring the early litters. This allele favors devoting more resources to raising the current litter, as opposed to producing the next litter. This manifests as an apparent grand-paternal preference for enhanced maternal care. Thus, it is possible to understand the selective factors favoring imprinting of genes that affect maternal care in terms of their inclusive-fitness effects, even in a context that is quite different from the one that we normally associate with imprinting. For most other phenotypes, however, understanding the evolution of imprinting will require a degree of understanding of complex molecular, physiological, and social systems that is not yet available.

2. Tissue-specific imprinting

As patterns of imprinted gene expression are studied in more detail, it is becoming clear that many loci are imprinted in a tissue-specific manner. In some eutherian mammals, including mice, rats, and cows, X-chromosome inactivation is random in fetal and adult somatic tissues, but is imprinted in extraembryonic tissues, where the paternally derived chromosome is specifically inactivated (see Lee, 2003; Reik and Lewis, 2005 for recent reviews). Many autosomal genes are also imprinted only in specific tissues or cell types, including *GRB10* (Blagitko et al., 2000; Hitchins et al., 2001), *Calcr* (Hoshiya et al., 2003), *Igf2/H19* (Charalambous et al., 2004), *UBE3A* (Rougeulle et al., 1997; Vu and Hoffman, 1997), ATP10A (formerly ATP10C) (Meguro et al., 2001; Herzing et al., 2001) and KCNQ1 (Gould and Pfeifer, 1998; Paulsen et al., 1998).

One of the most striking examples of complex patterns of imprinting occurs at the *GNAS1* locus. In most tissues, this locus biallelically produces $G_s\alpha$, the alpha subunit of the stimulatory G protein (Campbell et al., 1994; Hayward et al., 1998a b). However, this protein is expressed specifically from the maternally derived allele in some tissues, including the thyroid, gonads, and pituitary gland (Hayward et al., 2001; Germain-Lee et al., 2002; Mantovani et al., 2002; Liu et al., 2003). The locus also produces three transcripts that share exons 2–13 with this biallelic transcript, but which each contain alternate versions of exon 1. The XLαs transcript encodes an extra large variant of $G_s\alpha$, is expressed primarily in the nervous system and in neuroendocrine tissues, and is derived exclusively from the paternally derived allele (Kehlenbach et al., 1994; Hayward et al., 1998a; Peters et al., 1999). The A/B transcript is paternally expressed in a broad range of tissues, but appears not to be translated (Ishikawa et al., 1990; Swaroop et al., 1991; Liu et al., 2000). A maternally expressed transcript, NESP55, incorporates an alternate first exon that contains an in-frame termination codon (Ischia et al., 1997; Hayward et al., 1998b; Peters et al., 1999). Thus, while it shares exons 2–13 with the $G_s\alpha$ and XLαs trancripts, it contains no overlapping amino acid sequence. Finally, there is a paternally expressed, non-coding antisense trancript that overlaps with NESP55 (Hayward and Bonthron, 2000; Wroe et al., 2000). Each of these transcripts appears to have an independent promoter region that contains sites where the silenced allele is methylated.

While we cannot presently construct realistic models for many postnatal phenotypes associated with imprinting, it is possible to undertake a certain level of analysis without reference to the specific phenotypic effects of any locus.

General results derived from this type of analysis might then shed light on tissue-specific patterns of imprinting at particular loci. In this paper I will consider an evolutionary model of a generic imprinted gene that is expressed in somatic tissues and affects some phenotype other than fetal growth. I will focus in particular on the conditions favoring reactivation of a silenced allele in a particular tissue type or cell lineage.

3. Interactions in the propagation and interpretation of imprinted gene expression

Recent theoretical work on genomic imprinting has extended the kinship theory beyond the conflict between maternally derived and paternally derived genes. A number of papers have highlighted the distinction between *imprinted* and *imprinting* genes (Spencer and Williams, 1997; Burt and Trivers, 1998; Reik and Walter, 2001b; Wilkins and Haig, 2002). Specifically, the establishment of DNA methylation in oogenesis and spermatogenesis involves an interaction between *trans*-acting factors (such as methyltransferases) and the *cis*-acting regulatory sequences that are being modified. The inclusive-fitness functions associated with the *trans*-acting factors differ from those associated with the *cis*-acting factors, giving rise to potential evolutionary conflicts.

Similarly, the propagation and interpretation of the epigenetic modifications at imprinted loci involve interactions between distinct genetic factions. In the early rounds of cell division following fertilization, the zygote is transcriptionally inactive, and the *trans*-acting factors in the embryo are largely maternal-store proteins, which represent the mother's inclusive fitness (see Wilkins, 2005). Recent evidence indicates that certain enzymic activities may also be contributed by the incoming sperm, including phospholipase C (Swann et al., 2004) and 5-methylcytidyl deaminase (Jost et al., 2002), suggesting that there may be some small number of *trans*-acting factors in the early embryo that are evolving according to the father's inclusive fitness. Following transcriptional activation, the *trans*-acting factors involved in these processes are predominantly expressed from unimprinted loci, and are therefore evolving according to an inclusive-fitness function that includes kin of both the maternally and paternally derived alleles.

I will follow the convention of using the terms *madumnal* and *padumnal* to refer to maternally derived and paternally derived alleles present in the focal individual, as distinct from *maternal* and *paternal*, which refer to alleles expressed in that individual's mother and father. I will use the term *filial* to refer collectively to the madumnal and padumnal alleles in the focal individual. Alleles at an unimprinted locus in this individual are under selection to maximize the filial inclusive fitness, which is the sum of the matrilineal and patrilineal inclusive-fitness functions associated with the madumnal and padumnal alleles at an imprinted locus.

4. The model

I will use an extremely simple model of development in which an individual has a single embryonic tissue type (tissue 0), which differentiates into various adult somatic tissues. I will consider an imprinted locus whose expression is limited to the embryonic tissue and two somatic tissues (tissues 1 and 2). The expression strategy of an allele at this locus can then be described by the vector $[x_0^m, x_0^p, x_1^m, x_1^p, x_2^m, x_2^p]$, where the numeric subscript indicates the tissue type, and the m and p superscripts refer to the madumnal and padumnal alleles, respectively. The total expression level in each cell type will be indicated by X_0 $(= x_0^m + x_0^p)$, X_1 $(= x_1^m + x_1^p)$ and X_2 $(= x_2^m + x_2^p)$. I will assume that there are only two phenotypes relevant to the evolution of imprinting at this locus: an embryonic phenotype Φ_0, which is a function of only X_0 $(\Phi_0 = \Phi_0(X_0))$, and an adult phenotype Φ_a, which is a function of the expression level in both adult somatic tissues $(\Phi_a = \Phi_a(X_1, X_2))$. I will also assume that the fitness consequences of changes in the two phenotypes are completely independent $(W(\Phi_0, \Phi_a) = W_0(\Phi_0) + W_a(\Phi_a))$.

For simplicity of presentation, I will restrict my analysis to a locus that is madumnally silent in the embryonic tissue $(x_0^m = 0; X_0 = x_0^p)$. All of the analysis that follows applies equally to a padumnally silenced locus, with the superscripts m and p reversed. The kinship theory predicts this pattern of imprinted gene expression if the level of expression X_0 favored by the padumnal allele (X_0^p) is higher than that favored by the madumnal allele (X_0^m). Assuming that the embryonic phenotype is a monotonically increasing function of X_0 $(\partial\Phi_0/\partial X_0 > 0)$, and that the embryonic components of the matrilineal and patrilineal inclusive fitnesses (W_0^m and W_0^p, respectively) are each unimodal functions of Φ_0, this implies that the optimal phenotype from the perspective of the padumnal allele $(\hat{\Phi}_0^p)$ is higher than that for the madumnal allele $(\hat{\Phi}_0^m)$.

Because the goal of this work is to understand somatic reactivation, I will assume that the selective forces at the embryonic stage are constant, and will consider different scenarios of how the adult phenotype Φ_a is affected by somatic expression (X_1 and X_2). Without loss of generality, I define the quantitative measure of adult phenotype in such a way that madumnal alleles will favor a lower value than will padumnal alleles. Assuming, as above, that the inclusive fitnesses W_a^m and W_a^p are both unimodal functions of Φ_a, the phenotype that maximizes the filial (unimprinted) inclusive fitness W_a^f will lie between the madumnal and padumnal optima, so that

$$\hat{\Phi}_a^m < \hat{\Phi}_a^f < \hat{\Phi}_a^p. \tag{1}$$

In the absence of epigenetic reprogramming during development, the expression pattern at such a locus would be $[0, x_0^p, 0, x_1^p, 0, x_2^p] = [0, X_0, 0, X_1, 0, X_2]$. *Cis*-acting regulatory elements associated with the locus will then be under selection only when paternally derived, and expression is

expected to evolve to maximize W^p, so that $\Phi_a(X_1, X_2) = \Phi_a(x_1^p, x_2^p) = \hat{\Phi}_a^p$.

I will now consider the conditions that could favor reactivation of the silenced madumnal allele in one or both adult somatic tissues. Reactivation requires epigenetic reprogramming, which implies an interaction between *cis*-acting regulatory regions associated with the madumnal allele itself (subject to the fitness function W^m) and *trans*-acting factors that are subject to the filial inclusive fitness $W^f = W^m + W^p$. Condition (1) implies that

$$\left.\frac{\partial W_a^m}{\partial \Phi_a}\right|_{\Phi_a=\hat{\Phi}_a^p} < 0, \tag{2a}$$

and

$$\left.\frac{\partial W_a^f}{\partial \Phi_a}\right|_{\Phi_a=\hat{\Phi}_a^p} < 0. \tag{2b}$$

That is, when the phenotype is at the padumnal optimum ($\Phi_a = \hat{\Phi}_a^p$), both the madumnal and filial genetic factions would benefit from changes that reduce the phenotypic value.

Considering, for the moment, a single tissue, the conditions required for the madumnal and filial factions to favor reactivation of the madumnal allele are

$$\left.\frac{\partial W_a^m}{\partial X_i}\right|_{\Phi_a=\hat{\Phi}_a^p} > 0, \tag{3a}$$

and

$$\left.\frac{\partial W_a^f}{\partial X_i}\right|_{\Phi_a=\hat{\Phi}_a^p} > 0, \tag{3b}$$

respectively. Combining these conditions with (2a) and (2b), we can see that madumnal reactivation will be favored if

$$\left.\frac{\partial \Phi_a}{\partial X_i}\right|_{\Phi_a=\hat{\Phi}_a^p} < 0. \tag{4}$$

This condition applies to both the madumnal and filial factions, suggesting an absence of conflict over somatic reactivation. If condition (4) holds, both the *cis*- and *trans*-acting elements will favor reactivation, and both will disfavor reactivation if condition (4) does not hold.

Condition (4)—applied separately to each tissue—leads to certain inferences about the relative phenotypic and selective consequences of changes in gene expression based on tissue-specific patterns of monoallelic and biallelic expression. Specifically,

$$[0, x_0^p, 0, x_1^p, 0, x_2^p] \Rightarrow \left.\frac{\partial \Phi_a}{\partial X_1}\right|_{\Phi_a=\hat{\Phi}_a^p} > 0 \text{ and } \left.\frac{\partial \Phi_a}{\partial X_2}\right|_{\Phi_a=\hat{\Phi}_a^p} > 0, \tag{5a}$$

$$[0, x_0^p, x_1^f, x_1^f, x_2^f, x_2^f] \Rightarrow \left.\frac{\partial \Phi_a}{\partial X_1}\right|_{\Phi_a=\hat{\Phi}_a^p} < 0 \text{ and } \left.\frac{\partial \Phi_a}{\partial X_2}\right|_{\Phi_a=\hat{\Phi}_a^p} < 0, \tag{5b}$$

$$[0, x_0^p, x_1^f, x_1^f, 0, x_2^p] \Rightarrow \left.\frac{\partial \Phi_a}{\partial X_1}\right|_{\Phi_a=\hat{\Phi}_a^p} < 0 \text{ and } \left.\frac{\partial \Phi_a}{\partial X_2}\right|_{\Phi_a=\hat{\Phi}_a^p} > 0, \tag{5c}$$

where Φ_a has been defined in such a way that condition (1) holds. The superscript f in (5b) and (5c) emphasizes the fact that the expression level is identical for the madumnal and padumnal alleles. A fourth possibility, somatic reactivation limited to tissue 2, is equivalent to (5c) after relabeling the two tissues.

To understand the implications of statements (5a) through (5c), recall the selective forces active in early development, where the padumnal allele favored a higher expression level ($\hat{X}_0^m < \hat{X}_0^p$), resulting in madumnal silencing and padumnal optimization ($x_0^p = X_0 = \hat{X}_0^p$ and $\Phi_0 = \hat{\Phi}_0^p$). In (5a) the fitness asymmetries in the embryo are preserved in the somatic tissues; that is, the padumnal allele continues to favor higher expression. In (5b) the expression preferences are reversed, and it is the madumnal allele that favors higher expression in adult somatic tissues.

Note that neither of these conclusions makes any statement about the nature of the phenotype Φ_a, or the means by which this phenotype has differential effects on matrilineal and patrilineal kin. While we might expect the embryonic phenotype Φ_0 to be related to fetal growth, Φ_a might describe a behavioral trait whose fitness effects are meditated through complex social structures.

The situation described by (5c) is interesting in that it entails a reversal of preference in tissue 1, but a maintenance of preference in tissue 2. This implies that changes in expression in the two cell types have antagonistic effects on the phenotype Φ_a. To illustrate how this might arise, I will describe a hypothetical scenario. Many imprinted genes with prenatal growth effects also modulate mitogenic activity in adult tissues (Murphy and Jirtle, 2003; Feinberg and Tycko, 2004). This suggests that the relative level of expression of an imprinted gene in two different tissues during development could influence the relative size of two tissues. This might affect the allocation of resources to different tasks (e.g. gathering food versus finding mates). If different behavioral tasks are associated with different fitness consequences for matrilineal and patrilineal kin, then a conflict could arise between the madumnal and padumnal alleles over the allocation of resources (e.g. time and energy) to the different tasks—and therefore, to the different cell types.

This hypothetical scenario bears some similarity to observations from chimeric mouse embryos consisting of normal (biparental) cells and either parthenogenetic or androgenetic cells. These chimeras indicate differential contribution of maternally and paternally derived alleles to various brain structures (Allen et al., 1995; Keverne et al., 1996). Parthenogenetic chimeras have brains that are enlarged in the cortex and striatum, and the parthenogenetic

cells (which contain two madumnal alleles at each locus) contribute preferentially to these structures. Androgenetic chimeras have an enlarged hypothalamus, which contains an overrepresentation of androgenetic cells (with two padumnal alleles). These chimeras are suggestive of a conflict between madumnal and padumnal genes over the relative distribution of resources among various parts of the brain during development.

5. Predictions of the model

This model makes predictions regarding the inclusive-fitness consequences of tissue-specific changes in the expression level of genes that are imprinted early in development. It is worthwhile to make these predictions explicit, although they will be difficult to test in practice. The model also makes predictions about the relative phenotypic consequences of maternally and paternally inherited loss-of-function mutations. These predictions are in some ways counterintuitive and, more importantly, potentially testable.

If a locus that is madumnally silent in tissue 0 continues to be monoallelically expressed in tissue i, the model predicts the sign of the fitness effect of a small change in expression $X_i \to X_i + \Delta X_i$. Assuming expression at the padumnal optimum ($\Phi_a(X_i) = \Phi_a(\hat{X}_i) = \hat{\Phi}_i^p$):

$$\Delta W^m = \left.\frac{\partial W^m}{\partial X_i}\right|_{\Phi_a=\hat{\Phi}_a^p} \Delta X_i + O(\Delta X_i^2) \text{ therefore } \begin{array}{l} \Delta W^m > 0 \text{ if } \Delta X_i < 0, \\ \Delta W^m < 0 \text{ if } \Delta X_i > 0, \end{array} \quad (6a)$$

and

$$\Delta W^p = \left.\frac{\partial^2 W^p}{\partial X_i^2}\right|_{\Phi_a=\hat{\Phi}_a^p} \Delta X_i^2 + O(\Delta X_i^3) \text{ therefore } \Delta W^p < 0. \quad (6b)$$

That is, the matrilineal inclusive fitness W^m should increase if expression is reduced, and decrease if expression is raised. The patrilineal inclusive fitness W^p will be reduced by any change in expression.

Reactivation and biallelic expression will evolve only if the madumnal optimum for expression exceeds the padumnal optimum. Once biallelic expression arises it will evolve to the filial optimum ($X_i = X_i^f = 2x_i^f$). In the absence of a subsequent change in the ordering of the fitness optima, this implies that

$$\Delta W^m = \left.\frac{\partial W}{\partial X_i}\right|_{\Phi_a=\hat{\Phi}_a^f} \Delta X_i + O(\Delta X_i^2) \text{ therefore } \begin{array}{l} \Delta W^m > 0 \text{ if } \Delta X_i < 0, \\ \Delta W^m < 0 \text{ if } \Delta X_i > 0, \end{array} \quad (7a)$$

and

$$\Delta W^p = \left.\frac{\partial W^p}{\partial X_i}\right|_{\Phi_a=\hat{\Phi}_a^f} \Delta X_i + O(\Delta X_i^2) \text{ therefore } \begin{array}{l} \Delta W^p < 0 \text{ if } \Delta X_i < 0, \\ \Delta W^p > 0 \text{ if } \Delta X_i > 0. \end{array} \quad (7b)$$

Statement (7a) is identical to (6a). Statement (7b), however, indicates that small changes increasing expression from the locus should increase the patrilineal inclusive fitness W^p, in contrast with (6b).

In terms of the phenotypic consequences of an inherited loss-of-function mutation, (5a) and (5b) describe situations similar to those traditionally associated with imprinted and unimprinted genes. For (5a), mutations will have an effect only when paternally inherited, in which case the loss of function will be dominant. In (5b), the effect of a mutation on Φ_a will be independent of its parent of origin.

In (5c), where biallelic expression is restricted to tissue 1, theory predicts that expression in tissue 1 will evolve toward the filial optimum $\hat{\Phi}_a^f$, and expression in tissue two will evolve toward the padumnal optimum $\hat{\Phi}_a^p$. As I have described the model, it will not be possible to achieve both phenotypic optima simultaneously. This raises the possibility of an escalating arms race between the two. Increases in X_1 would reduce Φ_a to the benefit of W^f, and increases in X_2 would increase Φ_a to the benefit of W^p. This escalation would be limited either by functional constraints on the realizable expression level in one of the two tissues, or by pleiotropic deleterious effects of increased expression. The equilibrium phenotype would lie within the region bounded by the two optima: $\hat{\Phi}_a^f \leqslant \Phi_a \leqslant \hat{\Phi}_a^p$, with the exact value determined by the relative magnitudes of the functional and/or selective constraints in the two tissues. We should expect this equilibrium to lie closer to the optimum associated with the less constrained of the tissues.

Within this context, it is possible to consider the expected phenotypic effects of maternally and paternally inherited mutations. In particular we can imagine a loss-of-function mutation that effectively reduces the expression level of an allele to zero. If the wild-type expression pattern is $[0, x_0^p, x_1^f, x_1^f, 0, x_2^p]$, then with maternal and paternal inheritance, the mutant expression patterns would be $[0, x_0^p, 0, x_1^f, 0, x_2^p]$ and $[0, 0, x_1^f, 0, 0, 0]$, respectively. In both cases, X_1 is reduced by 50%. Maternal inheritance of this mutant has no effect on X_2, whereas paternal inheritance results in a functional knockout of the gene in tissue 2.

A naïve analysis might suggest that, whatever the phenotypic consequences of the mutation, the severity of the phenotype should be greater when it is paternally inherited. In the absence of intragenomic conflict, this intuition might be warranted. After all, the paternally inherited form has the consequences of the maternally inherited form, *plus* an additional effect that is not found under maternal inheritance. However, if the two tissues differ in imprinting, the associated knockouts will have

opposite phenotypic effects. Specifically, if we define $\Delta\Phi^f_{a1}$ as the change in phenotype associated with the 50% reduction in X_1, and $\Delta\Phi^p_{a2}$ as the change associated with the paternally inherited $X_2 \to 0$, then the phenotypic changes associated with maternal and paternal inheritance will be

$$\Delta\Phi^{MI}_a = \Delta\Phi^f_{a1}, \tag{8a}$$

and

$$\Delta\Phi^{PI}_a = \Delta\Phi^f_{a1} + \Delta\Phi^p_{a2}. \tag{8b}$$

Eq. (4) suggests that $\Delta\Phi^f_{a1} < 0$ and $\Delta\Phi^p_{a2} > 0$. If we loosely define the phenotypic severity of the mutation as the absolute deviation $|\Delta\Phi_a|$ from wild-type, the model predicts that it is in fact possible for the maternally inherited form to have the more severe phenotypic effects. Specifically,

$$|\Delta\Phi^{MI}_a| > |\Delta\Phi^{PI}_a| \text{ if } 2|\Delta\Phi^f_{a1}| > |\Delta\Phi^p_{a2}|. \tag{9}$$

Furthermore, if paternal inheritance is phenotypically more severe, it should have a phenotype that is in some sense opposite to the maternal phenotype. If the phenotypes associated with maternal and paternal inheritance are qualitatively similar (meaning that $\Delta\Phi_{MI}$ and $\Delta\Phi_{PI}$ have the same sign), then the maternally inherited phenotype should always be the more severe.

One potential difficulty facing experimental tests of these predictions arises from the gene's effect on Φ_0. For purposes of this analysis, I have assumed that the phenotypes Φ_0 and Φ_a are non-interacting. In real biological systems, however, this assumption will hold only in very special cases, if ever. In general, a mutation's effects on Φ_0 are likely to confound efforts to measure its effects on Φ_a. For example, in situation (5b), a mutation's effect on Φ_a is independent of the parent from whom the mutation was inherited. However, paternal inheritance might be associated with a significant fetal growth defect that is absent when the mutation is maternally inherited. There may be few adult phenotypes in reality that are unaffected by prenatal growth abnormalities.

Non-independence of Φ_a and Φ_0 also confounds the predictions for (5c). The prediction that phenotypic effects of mutation can be more severe when maternally inherited than when paternally inherited applies only to Φ_a. Maternally inherited loss-of-function mutations should have no effect on Φ_0. If adult loss-of-function phenotypes were always more severe when paternally inherited, it would be difficult to determine whether this falsified the predictions made here, or if these phenotypes reflected long-lasting developmental consequences of the effects on embryogenesis. More satisfactory tests of the model's predictions could be made if loss of function were limited to a particular tissue. Tissue-specific conditional knockouts for two DNA methyltransferases were recently constructed to examine gene function specifically in the germ line (Kaneda et al., 2004). In principle, similar techniques could be used to eliminate particular alleles from particular tissues, and to examine adult phenotypes in the absence of confounding effects on other phenotypes.

6. Independent phenotypes: GNAS

The scope of applicability of the conclusions presented here is limited by the assumption that the inclusive-fitness effects of gene expression in tissues 1 and 2 are mediated through a single phenotype Φ_a. If expression in these two tissues affect two distinct, non-interacting phenotypes, then the naïve predictions regarding the phenotypes of paternally and maternally inherited loss-of-function mutations would hold. That is, for a locus that is maternally silenced in some tissues and biallelically expressed in others, a maternally inherited loss-of-function mutation would manifest only through haploinsufficiency in the normally biallelic tissue. A paternally inherited loss-of-function would have this phenotype plus the knockout phenotype for the monoallelic tissue.

This multi-phenotype scenario likely applies to inherited mutations in the imprinted GNAS cluster. Mutations in $G_s\alpha$ are associated with pseudohypoparathyroidism (PHP), a collection of related disorders characterized by resistance to parathyroid hormone (PTH). For at least some of these mutations, the disease phenotype depends on the parent of origin. Paternal inheritance results in a condition called pseudopseudohypoparathyroidism (PPHP), which manifests as Albright's hereditary osteodystrophy (AHO). Maternal inheritance causes PHP type 1a, which is characterized by AHO, as well as PTH resistance. The primary GNAS transcript is biallelically expressed in some tissues and maternally expressed in others. The associated inheritance patterns suggest that the AHO phenotype results from haploinsufficiency in the biallelic tissues, while the PTH-resistance phenotype results from loss of function in tissues where this protein is maternally expressed. The relationship between the GNAS locus and the various forms of PHP has been reviewed recently by Bastepe and Jüppner (2005).

7. Deleterious mutations, the cost of imprinting, and epigenetic drift

In the preceding section, I have tried to explicitly describe the predictions that follow from the proposed selective basis for reactivation of biallelic expression at an imprinted locus. In particular, I have described the type of correlations that should be expected between tissue-specific patterns of imprinting and the phenotypic and fitness consequences of mutations if the selective processes described here have been the primary factor driving reactivation. To make these predicitons more meaningful, it is useful to briefly consider the patterns that might be expected under other scenarios.

One possibility is that there is no significant selection either for or against the reactivation of silenced alleles. Patterns of expression in adult tissues might be evolving

largely due to drift, or as a byproduct of other regulatory changes. It is difficult to make strong predictions regarding the pattern of reactivation expected under this scenario, but a strict neutralist hypothesis would suggest that a return to biallelic expression should be uncorrelated with gene function or tissue type. Such a lack of correlation might be discernible if tissue-specific reactivation of particular loci has occurred independently in multiple lineages.

A second possibility is that reactivation serves to protect the individual from the consequences of deleterious recessive somatic mutations. One potential fitness cost associated with imprinting derives from the fact that individuals are functionally hemizygous at imprinted loci, and are therefore subject to the full consequences of deleterious recessive mutations. For inherited mutations, this cost is extremely small, on the order of the mutation rate (Spencer and Williams, 1997), because deleterious mutations at an imprinted locus will segregate at low frequency at mutation-selection balance. However, somatic mutations could potentially represent a significant factor favoring reactivation of silenced alleles, and should be considered as an alternative hypothesis. I will consider in slightly more detail the patterns of reactivation suggested by this alternative, and how they might differ from those of the conflict-based explanation modeled in this paper.

Numerous imprinted genes have been linked to cancer (Murphy and Jirtle, 2003; Feinberg and Tycko, 2004). Typically, the mutations found in tumors are those that one might expect to be mitogenic: loss-of-function mutations at padumnally silent, growth-suppressing loci, and loss of imprinting (spontaneous reactivation) at madumnally silent, growth-enhancing loci (Hernandez et al., 2003). If the deleterious effects of somatic mutations are predominantly attributable to tumor growth, loci that are biallelically expressed in somatic tissues should be predominantly those with growth-suppressing functions. Assuming that prenatal growth suppressors suppress growth in any tissue, this would correspond to loci that are padumnally silenced during embryogenesis.

More generally, if deleterious somatic mutations drive reactivation, the loci and tissues in which we find biallelic expression should correlate with the magnitude of selection against loss-of-function mutations. This could most easily be tested using tissue-specific conditional knockouts for loci with tissue-specific reactivation (as in (5c)). Homozygous knockouts in tissues where the gene is biallelically expressed would be predicted to have a larger fitness effect than the same knockout in a tissue with monoallelic expression. A similar pattern would be expected within individual tissues, where knockouts of imprinted genes that have been reactivated should be more deleterious than knockouts of genes that continue to be monoallelically expressed.

Protection against deleterious recessive somatic mutations and the conflict-based model developed in this paper lead to different predictions regarding the correlation between gene expression patterns and phenotypic effects of somatic homozygous knockouts. Patterns observed across multiple tissues and loci could, in principle, be used to distinguish which of these two hypotheses is a better candidate for the predominant selective factor favoring transcriptional reactivation of imprinted genes. However, the two hypotheses make stronger differentiating predictions regarding the phenotype of heterozygous knockouts that could be informative at the level of individual loci. In order for protection against deleterious recessive mutations to be a significant selective force at a particular locus, common deleterious mutations must, in fact, be recessive. This hypothesis therefore predicts little or no phenotypic effect of heterozygous somatic loss-of-function mutations in tissues where the silenced allele has been reactivated. By contrast, the conflict-based hypothesis developed here requires there to be significant selective forces acting on the quantitative level of gene expression. This suggests that loss-of-function mutations should not be strictly recessive at these loci. Put another way, if loss of function of one of the two alleles has no phenotypic effect, there should be no basis for selection for or against allele-specific transcriptional silencing.

8. Metastability of expression patterns and lability of expression levels

I have argued that natural selection will favor transcriptional reactivation in a particular tissue only if the silenced allele favors a higher expression level in that tissue than the transcriptionally active allele does. One of the assumptions underlying this argument is that mutations that qualitatively alter the expression *pattern* at a locus are rare relative to mutations that quantitatively alter the expression *level* from an allele at that locus.

For example, consider our madumnally silenced locus, and assume that natural selection favors increasing the expression level from that locus in a particular tissue. For simplicity, also assume that there is no intragenomic conflict; that is, the madumnal and padumnal alleles both favor this higher expression level. The new level could be achieved by increasing the expression from the already-active padumnal allele. Alternatively, transcription could be reactivated from the madumnal allele. The assumption made here is that expression from the padumnal allele will evolve rapidly to the new optimal level, thereby removing any incentive to increase madumnal expression. Only in the case where the madumnal optimum is higher than the padumnal optimum will natural selection continue to favor substitutions that reactivate the madumnal allele.

That gene expression levels are labile is an intuition shared by many biologists. However, in recent years, we have also begun to see the accumulation of empirical evidence to support these intuitions. RNA microarray experiments have demonstrated that there is a large amount of variation in gene expression level segregating in natural populations, and the possibility of rapid

evolution of these expression levels (Townsend et al., 2003; Rifkin et al., 2003; Wray et al., 2003; Lemos et al., 2005).

9. De novo tissue-specific imprinting

All of the analyses presented here have been based on the assumption that if a locus is imprinted in one or more adult tissues, it must also be imprinted in fetal tissues. Furthermore, I have assumed that selection for imprinting in fetal tissues is primary, and that tissue-specific imprinting evolves in the context of this preexisting imprint. These assumptions are motivated partly by mechanistic considerations, and partly by the taxonomic and functional distributions of imprinted loci. I believe that these assumptions are likely to be valid for many of these loci, but it is worth considering explicitly the circumstances under which they are likely to be violated.

The mechanistic argument is simply that two alleles can only be treated differently (e.g. receive different epigenetic modifications) if they can be distinguished. The physical separation of the proto-padumnal and proto-madumnal alleles in the male and female germlines, respectively, provides just such a mechanism for differential treatment of these two sets of alleles. If we consider each locus in isolation, unimprinted loci carry no information about their parent of origin, and therefore provide no basis by which differential modification or expression could be established.

Of course, imprinted genes do not exist in isolation, but rather inhabit chromosomes that contain many other genes, and differential epigenetic marks at one locus could be used as a basis for establishing differential marking of nearby loci. In fact, most imprinted genes occur in physical clusters along the chromosome (Verona et al., 2003), and epigenetic reprogramming is a dynamic process that continues after fertilization. For many imprinted gene clusters, there appears to be a relatively small "imprinting control element" that is differentially modified during gametogenesis (see Delaval and Feil, 2004; Smith et al., 2004; Soejima and Wagstaff, 2005). These small epigenetic asymmetries are translated into patterns of epigenetic differentiation spanning many loci, in some cases, quite late in development (Pickard et al., 2001; Umlauf et al., 2004). This secondary process does not rely on the same physical separation required for establishment of the primary imprint, but rather on mechanisms that propagate epigenetic modifications in *cis*.

The other rationale for these assumptions is based on the phenotypic and taxonomic patterns associated with imprinting. The kinship theory applies in principle to any locus where changes in the expression level have asymmetric effects on matrilineal and patrilineal kin. It could be argued that, in fact, there is no locus whose expression affects matrilineal and patrilineal kin *exactly* equally. However, in the vast majority of cases, the inclusive-fitness asymmetries associated with changes in expression may be so small that there is functionally no selection for imprinting.

The case of the loci that affect the distribution of maternal resources appears to be the primary exception. In organisms where offspring develop in physical contact with one of the two parents, there are strong inclusive-fitness asymmetries associated with loci that modulate demand on that parent. It is possible that this is the only context in which selection for imprinting is strong enough to overcome the barriers (either selective or inertial) that maintain biallelic expression at the vast majority of loci and in the vast majority of species. Consistent with this, among vertebrates, imprinting appears to be restricted to marsupials and eutherian mammals, and fetal-growth effects have been associated with most imprinted genes. Imprinting has also been observed in angiosperms, where the endosperm forms an interface between parent and offspring.

Once a locus arrives at monoallelic expression, however, selection for modifications (of expression level, pattern, or timing, or of the function of the gene product) will be directed solely by the inclusive fitness of the expressed allele. An imprinted gene might then acquire other characteristics beneficial preferentially either to the organism's matrilineal or patrilineal kin. The assumption being made here is that this subsequent selection on non-fetal-growth phenotypes is not sufficiently strong to drive the imprinting of an unimprinted gene, but may be strong enough to drive modifications at loci that are already imprinted.

A consequence of these assumptions is that if an imprinted gene has a non-growth phenotype, there should also be a growth phenotype associated with the gene. In cases where these assumptions are violated, it might be possible for de novo tissue-specific imprinting to arise. For example, *cis*-acting regulatory elements associated with a cluster of imprinted genes might elicit epigenetic modifications at a nearby locus, but only in one particular tissue. In this scenario, imprinting at that locus would be directly selected on the inclusive-fitness consequences of this modification in this particular tissue, and biallelic expression in other tissues would not require a selective explanation. At such a locus, the pattern of fitness and phenotypic consequences predicted here would not be expected to hold.

10. Concluding remarks

Imprinted gene expression has evolved as a result of evolutionary conflicts among the genes within an individual organism. Most theoretical work on imprinting has focused on conflicts in early development, and the epigenetic modifications in the parental germ lines that are driven by those conflicts. However, these imprinted genes are inherited by every cell in the body, and intragenomic conflicts over expression can persist into adulthood. In principle, any phenotype could be subject to

such a conflict, depending on the physiological, environmental, and social details of the system.

I have described a simple model that I hope will be helpful for interpreting future empirical results in the context of these ongoing intragenomic conflicts. I have described the conditions under which the reactivation of the silenced allele at an imprinted locus would be favored on the basis of inclusive-fitness considerations. The model was developed specifically with reference to an imprinted locus that is maternally silenced in the embryo. In terms of the somatic interactions among the madumnal, padumnal and filial genetic factions, the model is completely symmetrical, and its conclusions hold equally for a locus that is paternally silenced in embryogenesis. Similarly, although I have used terminology that implies a mammalian system (such as "fetal"), the arguments presented here should apply to other taxa with imprinted gene expression, such as plants.

Based on inclusive-fitness considerations, activation of a silenced allele will be favored only if there is a reversal in the direction of the intragenomic conflict relative to the conflict favoring imprinting in the germ line. This model is contrasted with another plausible explanation for reactivation—defense against deleterious recessive somatic mutations. The two models make different predictions with respect to the phenotypic and fitness effects of mutations in genes that are subject to reactivation.

Other predictions of the model presented here could be tested much more easily, by comparing the magnitude and severity of maternally and paternally inherited mutations. In particular, the model predicts (perhaps counterintuitively) that for a gene exhibiting tissue-specific imprinting, the somatic phenotypic effects of an inherited mutation may be greater when the mutant allele is silenced during development. The difficulty in testing this prediction is that inheritance of a mutation in the allele that is transcriptionally active in early development will have at least two phenotypic effects—one in the embryo and another in somatic tissues. There may be particular cases where these effects could be disentangled, but in general, the comparison will be difficult. Furthermore, in most cases the "adult phenotype" will actually be a complex set of phenotypes whose relation to each other may not be entirely transparent. However, as new technologies continue to be developed, such as the ability to construct tissue-specific conditional knockouts, it may be possible to explicitly test the predictions of alternative evolutionary models.

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