Metastable epialleles in mammals

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There are some mammalian alleles that display the unusual characteristic of variable expressivity in the absence of genetic heterogeneity. It has recently become evident that this is because the activity of these alleles is dependent on their epigenetic state. Interestingly, the epigenetic state is somewhat labile, resulting in phenotypic mosaicism between cells (variegation) and also between individuals (variable expressivity). The establishment of the epigenetic state occurs during early embryogenesis and is a probabilistic event that is influenced by whether the allele is carried on the paternal or maternal alleles. In addition, the epigenetic state determines whether these alleles are dominant. We propose that mammalian alleles with such characteristics should be termed metastable epialleles to distinguish them from traditional alleles. At this stage, it is unclear how common these alleles are, but an appreciation of their existence will aid in their identification.

For more than half a century, geneticists have been fascinated by mammalian alleles that are VARIABLY EXPRESSED (see Glossary), even in the absence of genetic heterogeneity. Two notable examples are the murine *agouti viable yellow* (A^{vy}) [1–3] and *axin fused* ($Axin^{Fu}$) [4,5] alleles. A list of others known to date is shown in Table 1. Despite the fact that such alleles have been known for so long, their characteristics are such that it is difficult to categorize them using conventional genetic terminology. It is now clear that the variable expressivity arises because the activity of these alleles is dependent on their epigenetic state [2,6–11]. For example, isogenic mice that carry the A^{vy} allele display a range of coat colours from completely yellow to wild-type agouti [2]. A yellow coat correlates with a hypomethylated, and therefore active, EPIALLELE. Similarly, $Axin^{Fu}$ mice have a spectrum of tail phenotypes, from severely kinked to completely normal, and the mutant phenotype, having a kinked tail, also correlates with a hypomethylated epiallele (V. Rakyan, unpublished). Such alleles, owing to their sensitivity to epigenetic state (in this case methylation) have several peculiar characteristics. For example, because the activity is dependent on epigenetic state, epiallelic variants actually differ in their relative dominance. These alleles are also sensitive to parental origin, although this effect is not the same as classic parental imprinting. Most importantly, the epigenetic state of these alleles is labile, resulting not only in variable expressivity, but also in VARIEGATION. This is reminiscent of position effect variegation (PEV) in *Drosophila* and plants where the activity of a translocated gene becomes sensitive to epigenetic effects because of its proximity to heterochromatin. We propose that mammalian genes that display these unusual properties should be referred to as METASTABLE EPIALLELES, which will reflect their true nature. In this article, we discuss the characteristics of these alleles that set them apart from more traditional alleles. Metastable epialleles could turn out to be more common than previously thought, and therefore contribute significantly to phenotypic diversity in mammals.

Relative dominance depends on epiallelic form

The concept of allelic dominance was, as we know, formulated by Mendel. Subsequently, it was found that many phenotypic traits did not exhibit simple dominant–recessive patterns, and these alleles were better described as SEMI-DOMINANT or CO-DOMINANT. However, the relative dominance of metastable epialleles cannot be described adequately using any of these terms if we do not take into account their epigenetic state. We can use the A^{vy} allele as an example to illustrate this point. The A^{vy} allele has an intracisternal A particle (IAP) insertion upstream of the *agouti* gene [3]. A cryptic promoter in the IAP can induce ectopic expression of Agouti, resulting in mice with yellow coats [3]. Hypermethylation at the IAP abrogates ectopic expression and these individuals have a wild-type agouti coat colour, and are indistinguishable from wild-type (A/A) mice [2]. A^{vy}/A mice that have a hypomethylated A^{vy} epiallele have a yellow coat, suggesting that A^{vy} is dominant over A (Fig. 1). However, A^{vy}/A mice that have a hypermethylated A^{vy} epiallele have an agouti coat because the hypermethylated epiallele is effectively a revertant to wild type, and in this case A^{vy} is no longer dominant

Glossary

Co-dominance: When the heterozygous phenotype is not the intermediate between homozygous genotypes but has the characteristics of both of the corresponding homozygous genotypes.
Epiallele: An allele that can stably exist in more than one epigenetic state, resulting in different phenotypes. The DNA sequence of different epialleles of a particular gene is unchanged; for example, classic parentally imprinted genes.
Incomplete penetrance: When the phenotype expected from a particular genotype is not always expressed. Incomplete penetrance is an extreme form of variable expressivity.
Metastable epialleles: An epiallele at which the epigenetic state can switch and establishment is a probabilistic event. Once established, the state is mitotically inherited.
Semi-dominance (also incomplete or partial dominance): When the phenotype of the heterozygous genotype lies in the range between the phenotypes of the homozygous genotypes.
Variable expressivity: Refers to genes that are expressed to varying degrees among different individuals. It can arise because of the action of unlinked genetic modifiers or differences in the epigenetic state.
Variegation: Mosaic expression of a particular phenotype among cells of the same cell type; for example, mottled coat colour.

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Table 1. Summary of metastable epialleles in mammals

Allele[a]	Endogenous/ transgene	Characteristic phenotype[b]	Variable expressivity[c]	Variegation	Parent-of-origin effects[d]	Retroelement association	Refs
A^{vy}	Endogenous	Coat colour, diabetes	Yes	Yes	Yes	IAP	[1,2]
A^{hvy}	Endogenous	Coat colour, diabetes	Yes	Yes	Yes	IAP	[7]
A^{iapy}	Endogenous	Coat colour, diabetes	Yes	Yes	Yes	IAP	[3]
$Axin^{Fu}$	Endogenous	Tail kink	Yes	?	Yes	IAP	[4,5]
Axial defects[e]	Endogenous	Neural tube defects	Yes*	?	Yes	?	[32]
Disorganization	Endogenous	Skeletal abnormalities	Yes**	?	No	?	[33]
RSVIgmyc	Transgene	Transgene expression in myocytes	Yes	?	Yes	?	[8]
TKZ751	Transgene	*LacZ* expressing somatic cells	Yes**	No	Yes	?	[9]
Mtalpha#7	Transgene	*LacZ* expressing erythrocytes	Yes*	Yes	No	L1	[10]
239B	Transgene	*LacZ* expressing erythrocytes	Yes*	Yes	Yes	?	[11]
BLG transgene (lines 7 and 45)	Transgene	β-lactoglobulin expression	Yes**	Yes	?	?	[23]
Star (fox)[e]	Endogenous	Piebald spotting	Yes*	Yes	Yes	?	[34]

[a]All alleles are murine except for star which is a fox allele.
[b]The endogenous alleles display a range of phenotypes. Only the most characteristic phenotype is listed.
[c]These effects have been observed in an inbred strain (no asterisk), closed colony (asterisk) or neither (double asterisk).
[d]The parent-of-origin effects are distinct from classic parental imprinting.
[e]These alleles display effects similar to metastable epialleles but have yet to be characterized at the molecular level.

over *A*. Furthermore, some yellow homozygous (A^{vy}/A^{vy}) mice can have one hypomethylated and one hypermethylated A^{vy} allele, suggesting the former epiallele is dominant over the latter!

Not surprisingly, the relative dominance of metastable epialleles is still an unresolved issue; sometimes they are described as dominant [3,12] and at other times as semi-dominant [13]. Because we now know that metastable epialleles are epigenetically sensitive, we can finally address the question of whether the hypomethylated metastable epiallele is dominant or semi-dominant. We can compare the range of phenotypes observed for mice, homozygous for hypomethylated epialleles, with mice heterozygous for the hypomethylated epiallele. If the epiallele is dominant we will see no difference, but if the homozygous mice are more severely affected, this would suggest semi-dominance. For example, if we were to observe a higher proportion of homozygous yellow A^{vy} mice with two unmethylated A^{vy} alleles than those with one methylated and one unmethylated allele, it would suggest that A^{vy} is semi-dominant. It has not been possible to perform such experiments in the past.

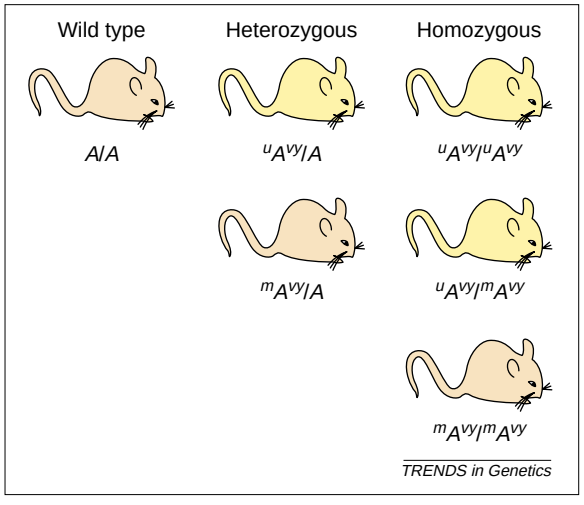

Fig. 1. The epigenetic state determines the relative dominance of metastable epialleles. Wild-type mice are (*A*/*A*) at the *agouti* locus and have an agouti coat. Heterozygous (A^{vy}/A) mice are yellow if they have an unmethylated A^{vy} allele ($^{u}A^{vy}$), and in this case the activity of A^{vy} is dominant over *A*. Heterozygous mice that are agouti carry a methylated A^{vy} allele ($^{m}A^{vy}$), which is effectively a revertant to wild-type and is therefore no longer dominant to *A*. Homozygous mice with yellow coats can carry two unmethylated A^{vy} alleles ($^{u}A^{vy}/^{u}A^{vy}$) or one unmethylated and one methylated A^{vy} allele ($^{u}A^{vy}/^{m}A^{vy}$). In the latter case, the unmethylated A^{vy} allele, is dominant over the methylated A^{vy} allele. Homozygous mice with two methylated A^{vy} alleles ($^{m}A^{vy}/^{m}A^{vy}$) are agouti.

Parent-of-origin effects at metastable epialleles

The parent-of-origin effects observed at metastable epialleles are quite different from those of classic parentally imprinted genes. Classic parental imprinting involves the epigenetic silencing of one allele of a gene, based on the parent of origin, resulting in monoallelic expression (Fig. 2). For instance, *Igf2* (*insulin-like growth factor 2*) is paternally expressed; that is, the paternally inherited allele is active, and the maternally inherited allele is inactive [14] (Fig. 2). At metastable epialleles, however, the epigenetic state is not strictly dependent on the parent of origin. For example, the probability of having a paternally inherited $Axin^{Fu}$ allele that is active is not 100%, as for *Igf2*, but only ~70% (V. Rakyan, unpublished) (Fig. 2). Furthermore, the probability of having a maternally inherited $Axin^{Fu}$ allele that is active is not 0% but ~40% (V. Rakyan, unpublished). Therefore, at metastable epialleles, the range of phenotypes shifts according to whether it has been inherited from the male or female parent. A similar type of effect has been observed previously for $Axin^{Fu}$ in a different strain background [5]. A hypothetical pedigree of a cross between $Axin^{Fu}$ homozygous mice with one active and one inactive allele (as observed at classic parentally imprinted genes), reveals that a range of phenotypes would be observed because some offspring would have two active alleles, some two inactive alleles, and some would have one active and one inactive allele (Fig. 2). Interestingly, in a few mice the 'imprinting' would actually be reversed; that is, the paternally inherited allele would be inactive and the maternally inherited allele would be active. Clearly this state of affairs is quite different and far more complex than what we observe at classic parentally imprinted genes.

The parent-of-origin effects at metastable epialleles and classic parentally imprinted genes might actually be the outcomes of different mechanisms. Current evidence suggests that at metastable epialleles, establishment of the parent-of-origin epigenetic differences could occur during early embryogenesis. This is based on the finding

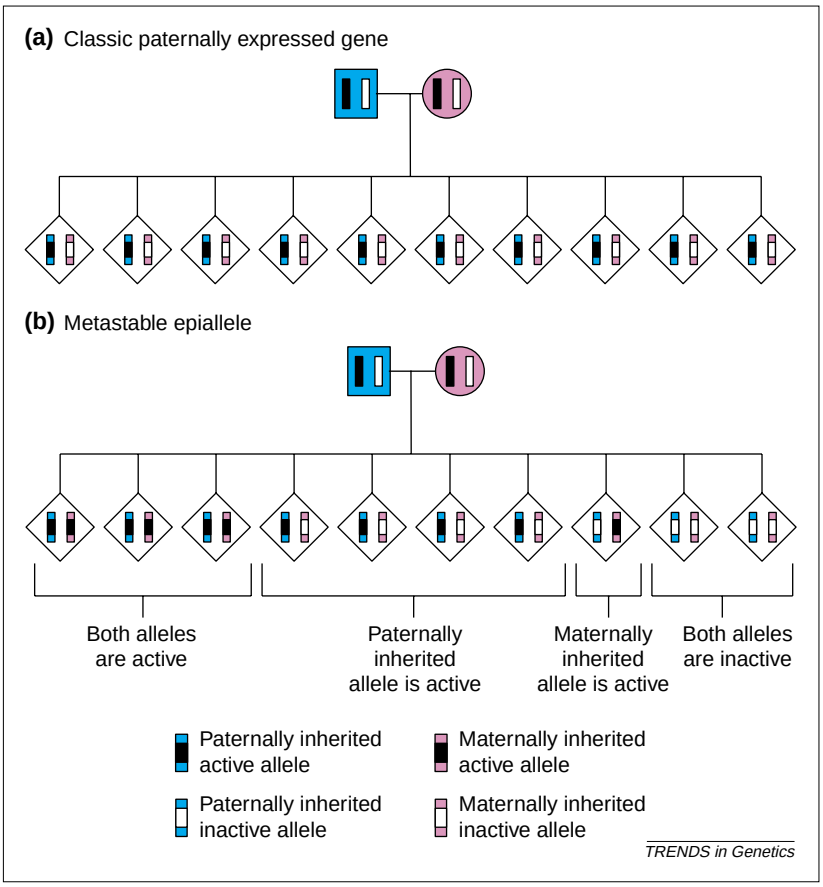

Fig. 2. Parent-of-origin effects at metastable epialleles are distinct from classic parental imprinting. In this diagram, paternally inherited alleles are blue and maternally inherited alleles are pink. Active alleles are indicated by black boxes and inactive alleles by white boxes. (a) All offspring display monoallelic expression from a classic paternally expressed gene because the probability of having a paternally inherited active allele is 100%, and the probability of having a maternally inherited active allele is 0%. There is no phenotypic variation among the offspring. (b) For the metastable epiallele *Axin*^{Fu}, the probability of having a paternally inherited active allele is ~70%, and the probability of having a maternally inherited active allele is ~40%. Consequently, the pedigree of a metastable epiallele is far more complex. Approximately 30% of the offspring would have two active alleles, 40% would have an active paternally inherited allele and an inactive maternally inherited allele, 10% would have an active maternally inherited allele and an inactive paternally inherited allele, and 20% would have two inactive alleles.

that, following the introduction of a different inbred strain at fertilization, parent-of-origin effects can be dramatically altered at metastable epialleles in the F1 offspring [1,5,8–10,15]. Indeed, it is now recognized that the paternal and maternal genomes can be differentiated, and treated differently with respect to methylation, post-fertilization [16–19]. At classic parentally imprinted genes, however, the differential epigenetic states are generally established during gametogenesis [20], and exposure to different genetic backgrounds rarely has a dramatic effect on the activity of the paternal or maternal alleles.

The epigenetic state at metastable epialleles is labile

The most distinctive characteristic of metastable epialleles is the labile nature of their epigenetic state. At most mammalian alleles, the genome-wide erasure and re-establishment of DNA methylation patterns during gametogenesis and early embryogenesis occurs in a predictable manner (reviewed in Refs [20,21]). At metastable epialleles, however, the re-establishment of epigenetic marks in early embryogenesis is a probabilistic event. This means that there is no guarantee that re-establishment will result in faithful restoration of the epigenetic state that existed before erasure. Differences in the re-established state between different cells of the same individual results in variegation, as evidenced by A^{vy} mice with mottled coats (i.e. patches of both yellow and agouti fur, [1–3]). Variegation of metastable epialleles has been elegantly demonstrated at the single-cell level using transgenic mice, where it was observed that a *lacZ* reporter gene was not expressed in all erythrocytes of an individual [10,11]. Differences in the re-established state between individuals results in variable expressivity; that is, A^{vy}/A mice can have yellow or agouti fur. It is highly unlikely that the variable expressivity of metastable epialleles is due to differences in unlinked genetic modifiers, as these effects are observed even when the alleles are studied in isogenic backgrounds [2,8,10,11]. At present, we do not know why the epigenetic marks are not faithfully restored, but it is possible that during re-establishment there are stochastic fluctuations in the levels of proteins that influence the epigenetic state at metastable alleles. Interestingly, at some metastable epialleles, the process of erasure is also somewhat inefficient and the epigenetic mark persists through meiosis, resulting in transgenerational epigenetic inheritance [1,2,4,5,10,11].

The association of metastable epialleles with retroelements and transgenesis

Interestingly, similar to the A^{vy} allele, *Axin*^{Fu} is associated with an IAP insertion [12], and it is likely that sensitivity of metastable epialleles to epigenetic modifications is due to their association with retroviral elements. In murine cells, most retroviral elements (e.g. IAPs, L1 elements) are thought to be maintained in a heavily methylated and transcriptionally inactive state [22–24]. However, it is clear that at certain retro-elements, the epigenetic state is metastable and can affect the activity of adjacent alleles. Consistent with this idea is the observation that the wild-type *agouti* and *axin* alleles do not display the unusual parent-of-origin effects or variable expressivity associated with A^{vy} and *Axin*^{Fu}. Several murine transgenes have also been found to be sensitive to epigenetic state and can be classified as metastable epialleles [8–11,25] (Table 1). This sensitivity could be because transgenes are recognized as foreign DNA insertions and are treated similarly to retroviral elements, or because they have integrated adjacent to retroviral elements and the epigenetic state spreads.

Why metastable epiallele?

We have chosen the word 'metastable' to emphasize the labile nature of the epigenetic state of these particular alleles. This word has been used for some time by plant biologists to describe alleles such as *pl*, *r* and *B*, where the activity of the allele is dependent on epigenetic state and the epigenetic state has a natural tendency to switch [26,27]. These plant alleles also show non-mendelian patterns of inheritance, such as paramutation [28].

The term epiallele has generally been used to describe alleles whose epigenetic state is transgenerationally stable. For example, the *Tg(13HBV)E36-Pas* transgene, when inherited paternally, is unmethylated, but maternal transmission of the transgene results in methylation and transcriptional silencing that cannot be reversed, even by subsequent paternal transmission [29]. So *Tg(13HBV)E36-Pas* is not metastable because the methylated epiallele does not revert to the unmethylated epiallele. More importantly, the establishment of the epigenetic state at this transgene is predictable, and not a probabilistic event. By combining the two terms, as in metastable epialleles, the reader is presented with a clearer picture of both the molecular nature of the allele and its phenotypic consequences.

It is important to point out that, although for all the examples of metastable epialleles studied so far hypomethylation is associated with gene activity, it is quite possible that for some as yet unidentified metastable alleles, the active epiallele is associated with hypermethylation. Indeed, it is also possible that some metastable epialleles have a chromatin-based epigenetic mark only.

Conclusion

Clearly metastable epialleles have properties that are very different from classic alleles. But how common are these alleles, particularly in humans? It is not easy to address this question owing to our extreme genetic heterogeneity. The effects of these alleles are easier to discern when differences in genetic and environmental factors, between individuals, are minimized. This is only possible in inbred animals maintained in a controlled environment. The best approach to identify metastable epialleles in humans, could be to study these alleles in monozygotic twins, where the genetic differences, at least, would be negligible. In the absence of any experimental evidence, it is not unreasonable to postulate that some phenotypic differences in humans could be due to the probabilistic establishment of epigenetic states at metastable epialleles, during early development.

Approximately 9% of the human genome is composed of retrotransposons. So, if even a small proportion of them display the effects described here, then their potential influence on phenotypic variation could still be large [30]! Metastable epialleles could turn out to be the explanation for some human diseases and phenotypic traits that display INCOMPLETE PENETRANCE and have unusual patterns of inheritance. In this regard, it has been observed that variation in the methylation status of autosomal allelic sites in human tissue can be transmitted through the germ line [31]. Variable expressivity in humans has generally been attributed to differences in quantitative trait loci between different individuals. We propose that in some cases, the variable expressivity could be due to a single locus expressed variably as described in this article. More metastable epialleles will undoubtedly be found in the future, and the identification of such alleles will be possible only if we have a good knowledge of their characteristics and an appropriate classification system. There is still a great deal to discover about these highly unusual, fascinating alleles.

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