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Review Article

The Hunt for the Epiallele

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Understanding the origin of phenotypic variation remains one of the principle challenges of contemporary biology. Recent genome-wide association studies have identified association between common genetic variants and complex phenotype; however, the minimal effect sizes observed in such studies highlight the potential for other causal factors to be involved in phenotypic variation. The epigenetic state of an organism (or 'epigenome') incorporates a landscape of complex and plastic molecular events that may underlie the 'missing link' that integrates genotype with phenotype. The nature of these processes has been the subject of intense scientific study over the recent years, and characterisation of epigenetic variation, in the form of 'epialleles', is providing fascinating insight into how the genome functions within a range of developmental processes, environments, and in states of health and disease. This review will discuss how and when mammalian epialleles may be generated and their interaction with genetic and environmental factors. We will outline how an epiallele has a variable relationship with phenotype, and how new technologies may be used for their detection and to facilitate an understanding of their contribution to phenotype. Finally, we will consider epialleles in population variation and their teleological role in evolution. Environ. Mol. Mutagen. 52:1–11, 2011.

Key words: epigenetics; epiallele; epigenome; phenotypic variation; DNA methylation

EPIALLELES

Epialleles are genomic regions at which the epigenetic state varies amongst individuals within a population. This variability can manifest, theoretically, through any one or more of a vast array of epigenetic modifications, including DNA methylation, post-translational modification of histones, or through other chromatin modifying factors. In turn, this variation can influence phenotype through downstream or bidirectional influences over gene expression [Zilberman et al., 2007]. Furthermore, epialleles must be stable through mitotic cell division. Rather than discussing epialleles in the context of specific epigenetic modifications, we will focus on general principles that define epialleles and their role in mammalian species.

First, it is important to consider how epialleles may arise. Recent evidence suggests that epialleles arise at the point where genetic, environmental, and stochastic events converge, providing the mechanism by which environmental or other 'stochastic' factors increase the plasticity with which a fixed genotype is translated (Fig. 1). Based on this model, Richards [2006] has proposed a useful classification scheme.

Obligatory Epialleles

These are generated as a direct consequence of genetic polymorphism and are independent of environmental or stochastic influences (Fig. 2). Epialleles of this type may be associated with a specific haplotype, in which case they are said to occur in cis. Such epialleles remain stable and conform to Mendelian inheritance patterns. Single-locus studies have identified obligatory epialleles for DNA methylation, where a single nucleotide polymorphism (SNP) is responsible for the creation or deletion of a CpG site [Heijmans et al., 2007; Kong et al., 2009]. Genome-wide approaches have identified further examples of SNP associated, allele-specific methylation that is more widespread [Kerkel et al., 2008; Schalkwyk et al., 2010]. Another recent study has mapped differentially methylated regions (DMRs) in the F1 generation from crosses of unique, inbred mouse strains, and reports that the majority of DMRs coincided with the underlying genotype [Schilling et al., 2009]. Similarly, it has been reported that local

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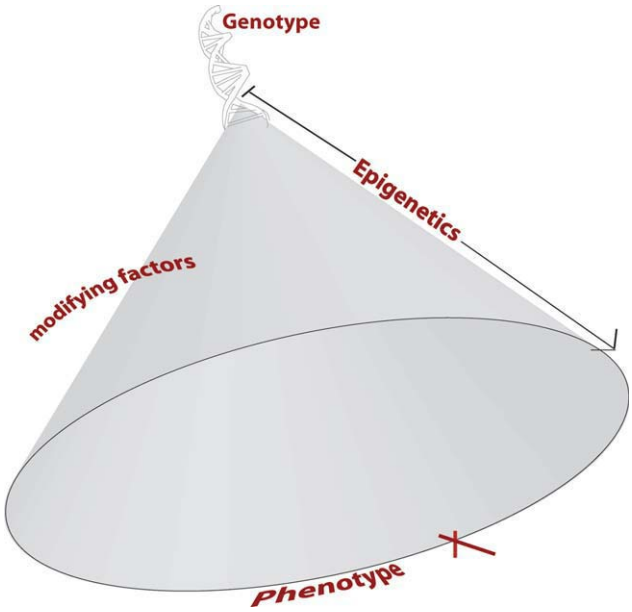

Fig. 1. The role of epigenetics in translating genotype into phenotype. A fixed genotype (apex) can give rise to many potential phenotypes, with any singe point on the directrix representing a potential phenotype (X). Epigenetics, represented here as the generatix of the cone, connects the genotype to the resultant phenotype in a deterministic manner. The position of the generatix may in turn be influenced by modifying factors, for example, diet. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

histone modifications may also segregate in a Mendelian manner, although the statistical power of this finding was weak [Kadota et al., 2007].

When an epiallele results from a polymorphism at a distant genomic location, it is termed trans-obligatory (Fig. 2). Epialleles of this nature are less easy to identify however, the mutagenesis screen utilised by Whitelaw and colleagues [Ashe et al., 2008; Blewitt et al., 2008] identified several chromatin modifiers using this principle. Mutations identified in their screen included core regulators of epigenetic phenomena, for example, DNA methyltransferase 1 (DNMT1) and, unsurprisingly, were associated with severe phenotypes, many being homozygous embryonically lethal [Blewitt et al., 2005]. Natural trans-obligatory epialleles would likely result from polymorphisms in factors that tether the core epigenetic machinery to specific alleles, producing much more localised epigenetic changes that would not be so strongly selected against. Supporting this view, it has been found that compound heterozygous or homozygous mutations in DNMT3B, which has a role in de novo DNA methylation, causes immunodeficiency, centromere instability, and facial anomaly (ICF) syndrome [Hansen et al., 1999; Xu et al., 1999]. A recent screen of DNMT polymorphisms in healthy humans revealed no non-synonymous polymorphisms in the catalytic domains of DNMT1/3A/3B, but

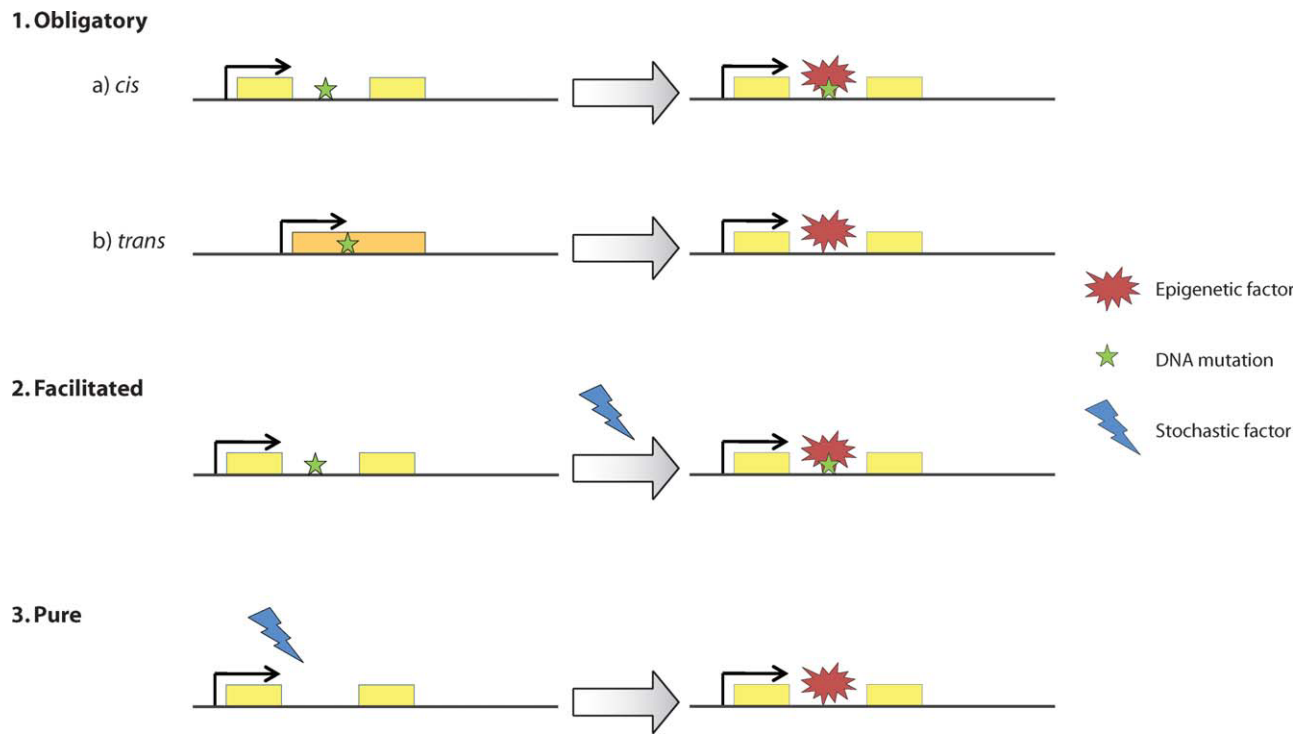

Fig. 2. Types of epialleles. (**1**) Obligatory epialleles. A genetic mutation (green star) either occurring at the site of the epiallele (red) (in cis), or at a distinct genomic location (in *trans*) is directly causative of epiallele formation. (**2**) Facilitated epialleles. A genetic mutation (green star) is a necessary substrate for epiallele (red) formation, but additional stochastic (blue bolt) factors are also required for epiallele formation. (**3**) Pure epialleles. Exist in the absence of any underlying genetic change and are derived as a result of stochastic factors (blue bolt) alone. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

identified only one polymorphism in DNMT3L that was associated with sub-telomeric hypomethylation when present in a heterozygous state [El-Maarri et al., 2009]. Trans-obligatory epialleles have also been recently implicated in the epigenetic deregulation that is characteristic of cancer; in this instance, somatic mutation of the H3K27 demethylase gene, *UTX*, was shown to influence the expression of genes regulated by Polycomb complexes and reintroduction of the functional protein into mutant cancer cell lines correlated with a reduced rate of cell proliferation [van Haaften et al., 2009].

Although obligatory epialleles influence phenotype through introducing epigenetic variation, they exist purely as a direct result of genetic variation. Whether alleles of this nature should be considered as epialleles is subject to ongoing debate.

Facilitated Epialleles

In other cases, genetic polymorphism may predispose to the formation of an epiallele without determining the epigenotype. Epialleles of this nature are termed 'facilitated', and their occurrence has been further defined as a severance in the direct link between genetic and epigenetic variation (Fig. 2) [Richards, 2006]. Facilitated epialleles, although mitotically stable, do not conform to Mendelian segregation. Some of the best examples of facilitated epialleles include the *agouti viable yellow* (A^{vy}) [Morgan et al., 1999] and *axin-fused* [Rakyan et al., 2003] alleles, both of which are associated with the insertion of an intracisternal-A particle (IAP) retrotransposon into a non-translated region of the endogenous gene. Promoters associated with these retrotransposons are capable of directing production of an aberrant transcript and are epigenetically regulated, potentially existing in a transcriptionally silent or active state. By contrast, in the absence of the IAP insertion, the endogenous gene does not exist as an epiallele, and thus the genetic event is seen to be a necessary substrate for its creation. These epialleles have been studied in isogenic mice, eliminating confounding effects of trans-modifiers and revealing that the epigenetic state of the introduced IAP promoter is stochastically established early in development [Morgan et al., 1999; Rakyan et al., 2003].

In humans, a possible example of a facilitated epiallele is observed in fragile-X syndrome, a condition associated with expansion of a repetitive CGG sequence in the 5′ untranslated region of the X-linked *FMR1* gene. This expansion predisposes to aberrant methylation and reduced *FMR1* expression, producing the Fragile X phenotype. Furthermore, in a family of five brothers all carrying the same predisposing X allele, methylation and FMR1 expression are mosaic and variably expressed amongst the brothers, producing phenotypes ranging from silent to fully penetrant [Stoger et al., 1997].

Pure Epialleles

These epialleles arise independently of genetic variation. In such cases, it is thought that environmental or 'stochastic' factors act to induce epigenetic variance, subsequently producing phenotypic plasticity [Fraga et al., 2005; Kaminsky et al., 2009] (Fig. 2). A range of specific environmental factors have been implicated, including diet and drug/toxin exposure, whilst in many cases, the inducer remains ill-defined. The concept that environment can induce epiallelic variance, with mitotic and/or meiotic stability, seems particularly relevant to elucidating the mechanisms of fetal programming. This hypothesis, based on a wealth of epidemiological data, associates adverse gestational environment with an increased risk of adult-onset disease (such as Type 2 diabetes or cardiovascular disease) [Barker, 1997; Sobngwi et al., 2003; Heijmans et al., 2008; Yajnik et al., 2008]. Animal models of maternal low protein show hypomethylation at the GR and PPARα gene promoters in offspring-examples of pure epialleles resulting from an early environmental insult. Further evidence for the epigenetic role in programming comes from the finding that supplementation with the methyl donor, folate, prevents the hypomethylation in response to low protein [Burdge et al., 2009]. The phenotypic consequence of exposure to low-protein diet is an insulin resistant phenotype in offspring [Burns et al., 1997; Lillycrop et al., 2008], replicating the observation from human cohorts of maternal famine exposure leading to excess risk of type 2 diabetes in offspring [Ravelli et al., 1998]. These comparisons raise a tempting possibility that human epigenomic studies may also enable the identification of pure epialleles.

WHEN ARE EPIALLELES GENERATED?

In mammalian development, two periods of genome-wide epigenetic reprogramming occur, during which time development-specific epigenetic marks are established. The first event is timed during the formation of germ cells (gametogenesis) and is required for the establishment of a unique germ-cell-specific gene expression signature, including the erasure and re-establishment of parental imprints and reactivation of the inactive X chromosome in preparation for meiosis [Sasaki and Matsui, 2008]. The second event occurs post-fertilisation, when maternally and paternally contributed genomes are processed differently; the male genome sees rapid replacement of its protamines with histones, and DNA methylation patterns are erased across male and female genomes by active and passive mechanisms, respectively [Morgan et al., 2005;Abdalla et al., 2009] (Fig. 3).

Incomplete erasure or variation in the re-establishment of epigenetic marks in either of these two rounds of reprogramming has the potential to give rise to epialleles. The creation of these epialleles during gametogenesis or

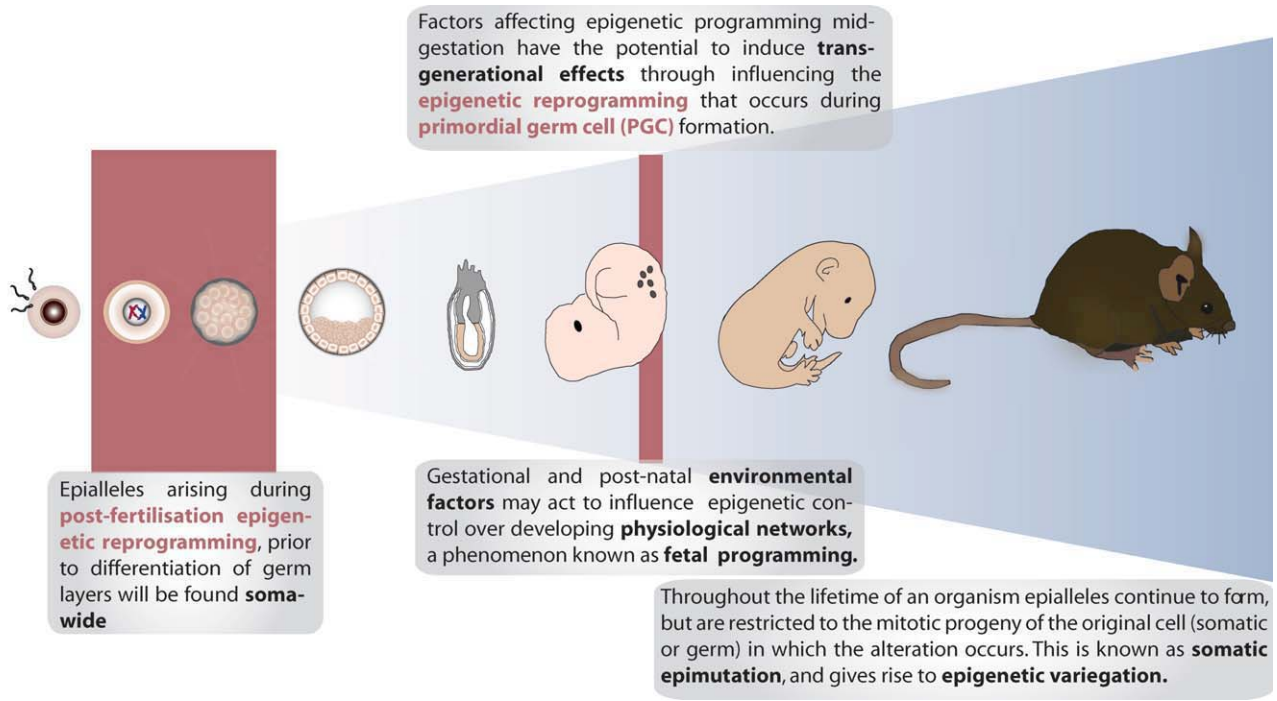

Fig. 3. The origins of epialleles. The developmental period at which an epiallele originates will determine how it might influence phenotype as well as the potential for it to be meiotically heritable. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

early embryogenesis results in their penetrance through all somatic lineages, exemplified by the human soma-wide epigenetic silencing of the mismatch repair gene, *MLH1* [Suter et al., 2004]. The potential for disruption of these complex processes by external influences is great. Adverse environmental factors, such as gestational diet and toxin exposure, have been found to induce epialleles during these susceptible developmental windows. For example, when pregnant dams carrying A^{vy}/a offspring are fed a methyl donor supplemented diet mid-gestation, not only the F1 show a shift in phenotype towards increased silencing of the A^{vy} epiallele, but also the F2, indicating that exposure of the developing germ cells in F1 to methyl donors influences the A^{vy} epigenotype in a manner that survives post-fertilisation reprogramming [Cropley et al., 2006].

Substantial evidence from epidemiological and animal studies to suggests that adverse environmental factors acting during fetal and early postnatal development can also have lifelong physiological consequences for the exposed individuals [Barker, 2004; Sinclair et al., 2007], through the phenomena of 'fetal programming' described earlier. However, at present, there is limited understanding of the relationship between the developmental stage of an organism, the induction of epialleles, and their interaction with underlying genetic susceptibility. The DNA methylation changes identified at candidate loci in offspring exposed to the Dutch Hunger Winter in utero have been identified as specific to periconceptual exposure [Tobi et al., 2009]. However, these methylation changes are small and, to date, no association with gene expression or phenotypic outcome has been described. However, more direct evidence for the involvement of epigenetics in the establishment of lifelong gene expression profiles in response to environmental factors has been provided by animal studies of both nutritional and behavioural models [Weaver et al., 2004; Cropley et al., 2006; Waterland et al., 2006].

Although gametogenesis and embryonic/fetal development represent the most dynamic periods of erasure and establishment of epigenetic marks in mammalian lifetimes, epialleles can continue to arise throughout the life of an organism. In such cases, epialleles will be restricted to the mitotic progeny of the cell in which the epigenetic alteration originated. The occurrence of somatic epimutation and its association with disease, especially cancer, is well documented [Feinberg, 2007]. The continued perturbation of the epigenome by the environment is further supported by the finding that the epigenomes of monozygotic twins become more dissimilar as the twins age [Fraga et al., 2005].

TRANSGENERATIONAL TRANSMISSION OF EPIALLELES

Some epialleles can be stably transmitted through meiosis and are therefore heritable, a phenomenon well

TABLE I. A Comparison of Techniques Available for Epiallele Identification

Epigenetic modification	Approach	Assay	Resolution	Quantitative	Relative cost	References
	Targeted	Bisulphite sequencing/ pyrosequencing	*****	**	$[a]	Frommer et al., 1992
		Methylation-specific PCR (if combined with qPCR)	***	*	$[a]	Colella et al., 2003
		Combined bisulphite restriction analysis	*	**		Xiong and Laird, 1997
		EpiTYPER™	***	****	$[a]	Docherty et al., 2009
DNA methylation	Genome-wide	Bead arrays	***	***	$$	Bibikova et al., 2006
		Methylation-specific restriction digestion-chip	**	***	$$	Lippman et al., 2005
		MeDIP-chip	**	**	$$	Weber et al., 2005
	Whole genome	RRBS-seq	*****	****	$$$	Meissner et al., 2008
		MeDIP-seq	**	**	$$	Down et al., 2008
		Shotgun bis-seq	*****	****	$$$$	Cokus et al., 2008; Lister et al., 2008; Lister et al., 2009
Post-translational histone modifications	Targeted	ChIP-qPCR	**	****	$[a]	Das et al., 2004
	Genome-wide	ChIP-chip	**	**	$$	Horak and Snyder, 2002
	Whole genome	ChIP-seq	**	***	$$$	Robertson et al., 2007

[a]Cost dependent on number of samples/regions assayed.

documented in plants, for example, *Linaria vulgaris* [Cubas et al., 1999], which lack the same germline-soma partitioning of mammals. Inbred animal models have provided some evidence for the meiotic transmission of non-obligatory epialleles in mammals, which, by definition, should be present in germ cells and resistant to the complex epigenetic reprogramming occurring post-fertilisation [Rakyan and Beck, 2006]. However, the existence of meiotically stable epialleles in human cohorts is difficult to demonstrate and remains controversial, although it has been inferred based on the detection of epialleles in the parents' gametes and the offspring [Suter et al., 2004]. As humans are genetically diverse, it is difficult to demonstrate the origin of any identified epialleles, which may result from genetic variation in trans-modifiers (i.e., as trans-obligatory epialleles). Animal models that describe transgenerational inheritance of environmentally induced epialleles also remain controversial as few studies have demonstrated transmission past the F2 generation, itself subject to the in utero environment as developing germ cells in F1 [Cropley et al., 2006), and the limited studies that have reported F3 effects have not being subject to successful independent replication [Chang et al., 2006, 2009].

IDENTIFICATION OF EPIALLELES

The early years of epigenetic research focussed on specific genomic locations found to influence gene expression, such as CpG islands within gene promoters. More recently, new technology has permitted the unbiased identification of epigenetic variants on an 'epigenomic' scale, coupled with the ability to simultaneously define the underlying genetic sequence. Here, we compare a range of experimental approaches to identify epialleles, taking into consideration study design, cost, and sample throughput (Table I).

Targeted Approaches

Targeted genomic approaches are hypothesis driven and are likely relevant to specific phenotype or disease models, enabling the fast and cost-effective interrogation of specific loci of interest. For resolution of DNA methylation, bisulphite sequencing is considered the 'gold standard'. Sodium bisulphite treatment of DNA results in the conversion of cytosine to uracil, permitting the distinction of unmethylated from methylated cytosines, because methylation protects from conversion. Coupled to PCR and sequencing, this provides a simple means of detecting both epigenetic and allelic information at relatively low cost [Frommer et al., 1992; Korshunova et al., 2008; Kroeger et al., 2008] and, therefore, has significant advantages over mass spectrometry-based techniques [Thompson et al., 2009]. In the case of histone marks, regions of interest can be assessed by quantifying the enrichment after chromatin immunoprecipitation (ChIP) with real-time PCR [Pietrobono et al., 2005; Tabolacci et al., 2008].

Genome-Wide Approaches

Genome-wide approaches assess epigenetic modifications at multiple pre-determined targets across the genome. In the case of DNA methylation, immunoprecipitation of

methylated DNA (MeDIP) [Weber et al., 2005] and methylation-sensitive restriction enzymes [Lippman et al., 2005] are used to isolate sites of methylation, the former by antibody enrichment and the second by restriction digest of CpG sites. In combination with commercial predesigned microarrays, the methylation-enriched sample can be hybridised to probes from genomic sites of interest, providing a relatively inexpensive high-throughput analysis of multiple locations across the genome [Rakyan et al., 2008]. Bead-array techniques, in combination with bisulphite conversion, offer an approach for allele-specific methylation detection at multiple CpG sites and are also suitable for multiplexed samples [Bibikova et al., 2006].

A similar genome-wide strategy can be used to identify histone modifications, combining ChIP with microarray (ChIP on chip) [Horak and Snyder, 2002]. Compared to DNA methylation, chromatin analysis is more expensive and time consuming, because only a single histone modification can be immunoprecipitated in each assay, necessitating multiple assays to map the likely interaction of different histone marks involved in the creation of an epiallele.

This range of experimental methods has been widely applied to investigate the role of epialleles in the pathogenesis of several human diseases, especially those for which molecular pathways and candidate genes have been well investigated. It seems likely that, with the increasing affordability of these genome-wide approaches, the detection of epialleles may soon be incorporated into cancer screening and staging [Feinberg et al., 2002] and biomarker detection [Zhang et al., 2009].

Whole Genome Approaches

The advent of high-throughput sequencing (HTS) techniques allows for powerful 'discovery-based' approaches that can be applied to the identification of epialleles at any genomic site. MeDIP-seq (MeDIP combined with HTS) provides a high-resolution platform for the quantitative identification of methylation differences across the entire genome; such differences between individuals providing examples of epialleles [Down et al., 2008]. However, MeDIP-seq is unable to provide single-base resolution of methylation patterns. Methylation-specific restriction digestion has also been combined with HTS, providing an alternative to MeDIP, with similar resolution [Brunner et al., 2009]. Whole-genome shotgun bisulphite sequencing (BS-seq) provides single-base resolution of DNA methylation across the genome [Cokus et al., 2008; Lister et al., 2008]. At the current time, the high cost of HTS and complex bioinformatic requirements negate its widespread use [Lister et al., 2009]. Reduced representation bisulphite sequencing provides a more pragmatic approach to BS-seq, using CpG-specific restriction digestion of genomic DNA combined with bisulphite conversion before HTS. However, this approach is limited by poor coverage of CpG-depleted regions [Meissner et al., 2008].

Likewise, the combination of ChIP with HTS, ChIP-seq, has been a successful approach in identifying chromatin variants in the human genome, generating high-resolution maps for up to 20 chromatin modifications [Barski et al., 2007; Robertson et al., 2007]. In the future, ChIP-seq will undoubtedly help in uncovering chromatin-based epialleles.

Powerful bioinformatic and statistical tools are integral to the success of whole-genome approaches in identifying epigenetic variation. The validation of results derived from such discovery-oriented experiments is also critical, and this should be performed in a larger sample size, using some of the genome-wide or targeted approaches described earlier (e.g., pyrosequencing or ChIP-qPCR).

Functional Analysis of Epialleles

To date, epiallelic variation is functionally characterised in terms of its influence over gene transcription. However, it is important to establish a deterministic link between an epiallele and altered gene expression, which could be influenced by unlinked genetic modifiers. Inbred mouse models provide a useful resource in which large numbers of genetically similar individuals can be simultaneously assessed for both the presence of the epiallele and its putative functional effect [Druker et al., 2004]. An integrative approach, combining several genome-scale data sets, offers the most powerful strategy to detect epialleles, which, in addition to cis effects, may exist monoallelically, or act by influencing distant enhancers. The power of combining epigenomic and transcriptomic mapping to detect epialleles with functional and phenotypic outcome is huge [Barski et al., 2007; Lister et al., 2008] and is likely to yield considerable data resources that could be made accessible publicly though browsers such as Ensembl. However, at present, cost and analytical barriers limit the potential to scale up these experimental approaches to the sample sizes that have given insight into complex disease pathogenesis from the detection of genetic variants [WTCCC, 2007].

Determining How Epialleles Are Generated

As previously discussed, the acquisition of an epiallele may be determined by the interaction of genetic and/or environmental factors. When designing an experimental approach to not only identify the presence of an epiallele, but also determine the factors that have led to its existence, appropriate study design is vital. Facilitated epialleles must be identified in the context of both genetic and epigenetic variation. Obligatory epialleles, which are induced solely through the existence of a genetic variant, may be detected by identifying the genetic variant or its

functional consequence in the first instance and the epigenetic event second [Bjornsson et al., 2008]. To study pure epialleles, an isogenic environment offers a means with which to increase the power to detect non-genetic events, and this has been successfully performed in inbred mouse strains [Pogribny et al., 2009] and studies of disease-discordant monozygotic twins [Javierre et al., 2010]. Identification of environmentally induced epialleles in human cohorts should also take careful note of potential confounding factors when considering their aetiological role [Wells, 2009].

EPIALLELES IN HEALTH AND DISEASE

In the recent years, numerous collaborative genome-wide association studies using large cohorts have yielded some insight into the genetic control of phenotypic diversity [HapMap, 2005; WTCCC, 2007]. Similar studies of the epiallelic contribution to complex traits have so far been limited by technological and economic factors as well as the analytical complexity required. However, some insight has been gained from the study of epiallelically induced phenotypic variation in monozygotic twins [Kaminsky et al., 2009] and the identification of pleiotropic quantitative loci, which account for phenotypic variance in mice [Cheverud et al., 2008]. Large-scale initiatives to develop a framework for the study of population epigenomics [Rakyan et al., 2004], together with open access data on standard genome browsers will foster further developments in this area [Rakyan et al., 2008]. Understanding the nature and extent of epiallelic effects on phenotype will likely prove relevant to the aetiology, prevention, and treatment of many human diseases.

For some time, it has been known that disruption of normal epigenetic regulation, for example, genomic imprinting can lead to developmental abnormalities. Parental imprinting occurs at $\sim$100 genes in humans [Bartolomei, 2009], resulting in parent-of-origin-specific monoallelic epigenetic silencing. Loss or gain of imprinting at these genes can be induced through either cis or trans-genetic or epigenetic processes. Beckwith–Wiedemann syndrome is an imprinting disorder of the *H19* imprinted cluster, in which both alleles are epigenetically silenced. In some cases, this has been shown to occur in the absence of any cis-genetic variation, suggesting that it might be an example of a pure epiallele; however, it cannot be excluded that a trans-genetic effect is responsible [Cerrato et al., 2008].

Many human cancers are characterised by genome-wide hypomethylation, with aberrant hypermethylation of CpG islands and subsequent silencing of the associated genes, including tumour suppressors [Momparler, 2003]. Inactivating mutation of mismatch repair genes predisposes to tumour initiation. Interestingly, epigenetic silencing of these genes in the absence of cis-genetic mutation has been found to correlate with the same tumour-susceptible phenotype [Suter et al., 2004; Chen et al., 2007]. However, further investigation is required to establish the relative contribution of both genetic and epigenetic factors in carcinogenesis. Recent evidence suggests that deregulation of Polycomb proteins, which are responsible for the co-ordination of development-specific epigenetic programming, may be responsible for the altered epigenetic profiles in cancer [Bracken and Helin, 2009].

Fetal programming of adult disease via environmentally determined epigenetic variants, with or without the association of genetic susceptibility, has been discussed earlier. To date, studies are limited and focus on individuals exposed to extreme environmental insults. Larger-scale studies encompassing milder environmental insults or behavioural patterns will increase our understanding of the complex interaction of epigenetic marks, genetic risk, and environment, and how they predispose to disease during an individual's lifetime. This knowledge is particularly pertinent given the increasing global prevalence of so-called complex diseases and, furthermore, may provide insight into how environmental factors may be modified as a means of disease prevention.

The identification of disease-associated epialleles either associated with disease induction, the disease process, has the potential to translate into screening, diagnosis, and identification of biomarkers [Shames et al., 2006]. An understanding of the epiallelic contribution to disease pathogenesis will also provide a platform for the development of targeted drug treatments. The role of epigenetic deregulation in cancer progression was identified serendipitously when pharmaceuticals such as the DNA methyltransferase inhibitors, 5-azacitidine, and 5-aza-2′ deoxycytidine were found to inhibit tumour growth [Von Hoff et al., 1976; Taylor and Jones, 1982; Kantarjian et al., 2006]. Now, newer more specific inhibitors have been developed with reduced toxicity [Datta et al., 2009]. An increased understanding of the origin and consequences of epigenetic deregulation in tumours will allow clinicians to predict responsiveness to such chemotherapeutic agents [Hegi et al., 2005]. Other pharmacotherapies now known to modulate epigenetic regulators (e.g., the anti-epileptic drug, valproic acid, a histone–deacetylase inhibitor) has enabled wider application to the treatment of cancer [Raffoux et al., 2005].

Evolutionary Aspects

There are several ways in which epialleles might influence evolutionary processes. It has been proposed that epigenetics might provide a mechanism for Lamarckian inheritance, described as the transgenerational inheritance of environmentally directed, adaptive, and phenotypic change [Jablonka and Lamb, 1995]. There are examples of environmentally induced epialleles in nature: the

honey bee (Apis Mellifera) uses nutritional-based programming of DNA methylation to determine the fate of fetal larvae as either worker or queen bees [Kucharski et al 2008], and inbred mouse models have shown environmentally directed modification of epialleles [Cooney et al 200, Waterland and Jirtle, 2003]. To date, transgenerational inheritance of epialleles is known to occur in plant species [Whittle et al., 2009]. However, transgenerational studies of environmentally induced epialleles in mammals have not yet provided conclusive evidence of their inheritance beyond F2, necessary to exclude the possible direct effects of the original exposure to F2 offspring via the F1 germline [Chang et al., 2006, 2009; Cropley et al., 2006].

Like genetic variation, inter-individual epiallelic variation does have the potential to influence phenotype, even if it is stochastic in origin. Epialleles can therefore be understood to increase further the phenotypic variation within a population beyond that which exists purely as a consequence of genetic variation. Recent population models have suggested that increased stochastic variation would be advantageous to a population in the face of shifting environmental pressures [Feinberg and Irizarry, 2009].

In addition to these stochastic influences on population variation, and their potential to induce environmentally directed adaptive evolution through an increased range of phenotypes on which natural selection can occur, epialleles may also be involved in facilitating genetic change. Methylation of cytosine residues facilitates their spontaneous deamination, resulting in an under-representation of CpG sites in the genomes of many species [Bird, 1980]. Relative hypomethylation of CpG islands in mammalian species or the absence of cytosine methylation in some invertebrates [Tweedie et al., 1997; Marhold et al., 2004] may reflect a protective function against this mutational decay. Recently, CpG methylation has also been linked with the rate of chromosomal rearrangement in a study comparing two hominids, the gibbon, and human [Carboneet al., 2009]. Whether epigenetic processes act in a broader sense to influence genome recombination rates, and therefore genetically driven evolutionary processes are, as yet, poorly understood.

Ongoing studies of epialleles and their potential for transgenerational inheritance will further elucidate the role of epiallelic variation in evolutionary processes. It is hoped that this understanding may shed light on how genetic and epigenetic variation interact to influence phenotypes across populations and in response to changing environmental pressures [Watve and Yajnik, 2007].

Perspectives

Conrad Waddington coined the term ‘epigenetics’ in the 1950s to refer to the process of how genotype gives rise to phenotype in development. Here, we define the epiallele as a region of the genome that varies with regard to its epigenetic state. Investigating the origin, prevalence, and contribution of epialleles to mammalian phenotypic variation is only now becoming accessible. Parallel genomic insights provide the basis for understanding epigenetic variation in the context of genetic variance. Rapid developments in technology offer researchers many platforms with which to study epialleles, from hypothesis-driven approaches to discovery-based methods.

In today’s fast-changing world, lifestyle and environmental impact on human health are increasingly relevant. As epialleles may arise where environment and genotype meet, clarifying their origin, windows of developmental susceptibility and phenotypic significance will provide greater insight into the biological mechanisms underlying many of the increasingly common ‘complex’ human diseases. Uncertainties around the existence of mammalian epigenetic inheritance remain, but careful study design and high-resolution technological methods, are likely to resolve these issues in the near future and provide insight into the role of epialleles in evolutionary processes. The hunt for the epiallele has only just begun. We have attempted to provide a contemporaneous definition of the epiallele and its potential role in a myriad of biological processes, the investigation of which will be the basis of many exciting scientific discoveries.

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