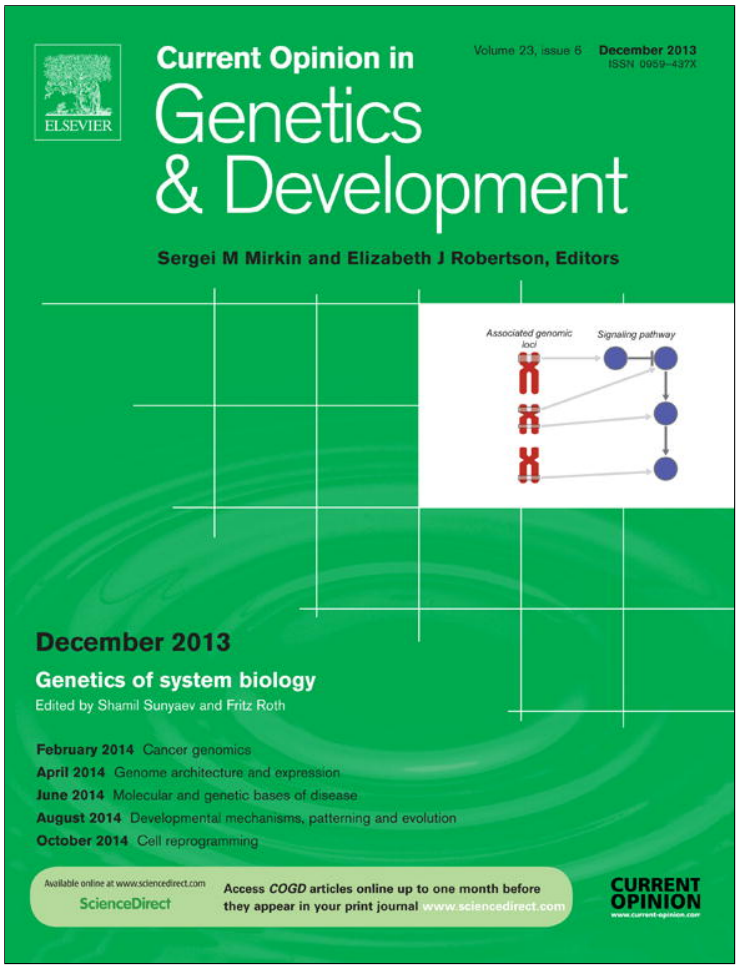

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Current Opinion in Genetics & Development

Autosomal monoallelic expression: genetics of epigenetic diversity?

Virginia Savova[1], Sébastien Vigneau[2,3] and Alexander A Gimelbrant[1]

In mammals, relative expression of the two parental alleles of many genes is controlled by one of three major epigenetic phenomena: X chromosome inactivation, imprinting, and mitotically stable autosomal monoallelic expression (MAE). MAE affects a large fraction of human autosomal genes and introduces enormous epigenetic heterogeneity in otherwise similar cell populations. Despite its prevalence, many functional and mechanistic aspects of MAE biology remain unknown. Several lines of evidence imply that MAE establishment and maintenance are controlled by a variety of genetic elements. Based on known genomic features regulating X-inactivation and imprinting, we outline likely features of MAE-controlling elements. We also assess implications of MAE for genotype–phenotype relationship, with a focus on haploinsufficiency.

Addresses
[1] Department of Cancer Biology, Dana-Farber Cancer Institute, Department of Genetics, Harvard Medical School, 450 Brookline Avenue, Boston, MA 02215, United States
[2] Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, United States

Corresponding author: Gimelbrant, Alexander A (gimelbrant@mail.dfci.harvard.edu, gimelbrant@genetics.med.harvard.edu)

[3] Present address: Department of Cancer Biology, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02215, United States.

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Introduction

From the systems level perspective, analysis of origins and consequences of cell-to-cell variability is essential to the understanding of biological processes as diverse as organogenesis, incomplete trait penetrance, and tumor evolution. During the course of development, various differentiation mechanisms create cells with deeply distinct morphologies, functions, and gene expression programs. A relatively neglected source of additional cell diversity is found in epigenetic mechanisms that differentially regulate the two parental copies of genes within the same cell type. In mammals, several major epigenetic mechanisms are involved in the generation of mitotically stable cell sub-populations through the separate regulation of each allele. Perhaps the best-known of these is X chromosome inactivation, which affects most X-linked genes [1] in female embryos: at the time of implantation, about half of the cells choose to inactivate the maternal copy of the X, while the rest inactivate the paternal X [2–4]. Another phenomenon is imprinting where regulation is uniform across cells [5]. The least understood phenomenon is *autosomal monoallelic expression* (MAE), which resembles X-inactivation in some ways but affects autosomal genes in both male and female cells.

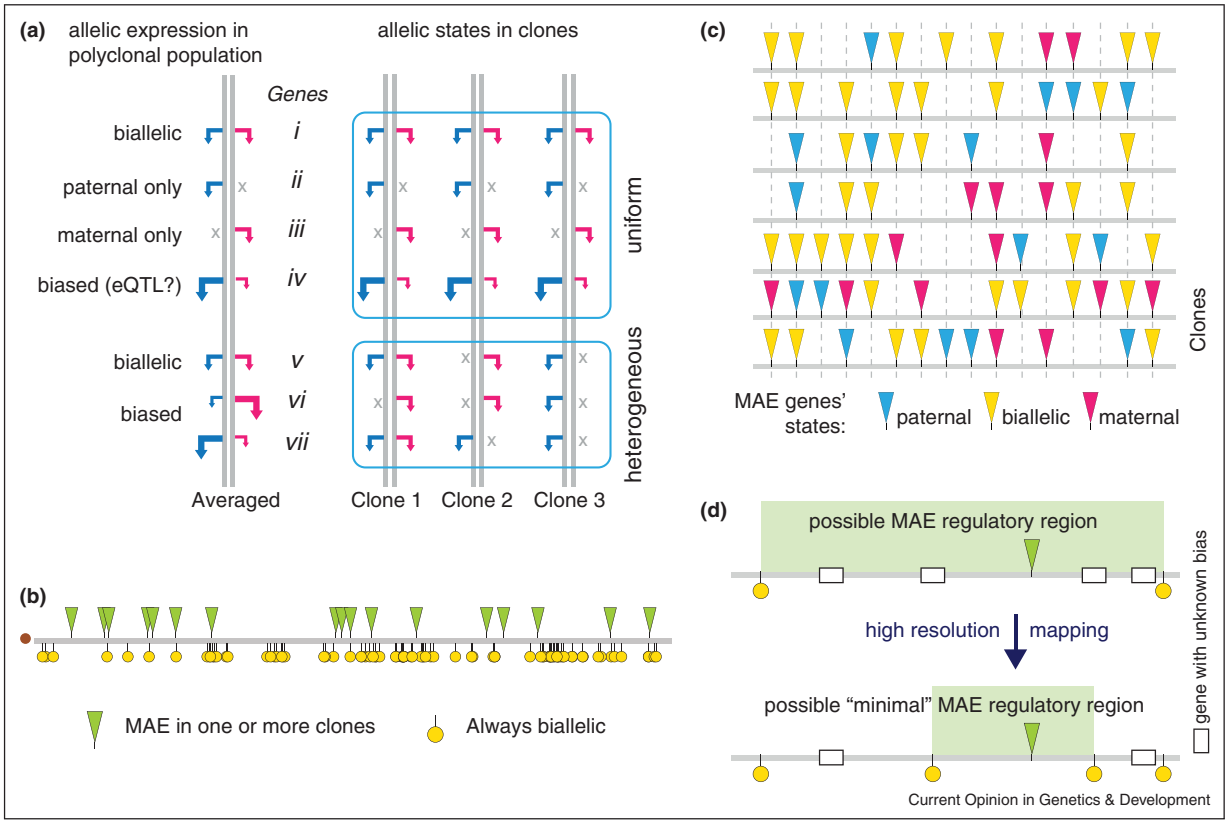
Here we review the current understanding of MAE biology and discuss functional consequences of cell-level heterogeneity introduced by MAE, as well as evidence for genetic mechanisms controlling its initiation and maintenance.

Clone-specific autosomal monoallelic expression

MAE can be defined as a mosaic epigenetic inactivation of one allele of an autosomal gene. Similarly to X-inactivation, some cells express the paternal allele, while other cells of the same type in the same individual express the maternal allele (Figure 1 and Box 1). The choice of the active allele, once made, appears to be maintained indefinitely. For example, mouse cells kept one copy of p120 catenin silenced after a year in continuous culture [6]. More generally, since systematic assessments of MAE have been performed on cell populations grown from a single cell to more than 10^7 cells [7,8•], we can conclude that the epigenetic allelic choices are maintained genome-wide through dozens of cell divisions. Because the allelic state of an arbitrarily chosen cell from a polyclonal population is not known ahead of time, MAE is sometimes called 'random'. We prefer another term, 'clone-specific' MAE, which avoids possible confusion with the transient differences resulting from transcriptional noise. In addition, it underscores the mitotic stability of MAE, as well as the heterogeneity of allelic choice.

The fraction of mammalian genes subject to MAE is surprisingly high. Allelic exclusion was discovered in immunoglobulins [9]. Later, MAE was found in olfactory receptors [10], which by themselves constitute about 5% of mammalian protein-coding genes, and also in some cytokines and other genes (reviewed in [11]). Recent systematic analyses of allele-specific expression in clonal

Figure 1



Widespread clone-specific monoallelic expression. **(a)** Relationship between allele-specific expression in polyclonal tissue and in individual clones. Both types of analysis are concordant when allelic expression patterns are uniform across cells (genes *i–iv*), but discordant when the cell population is heterogeneous (genes *v–vii*). In particular, clone-specific MAE is undetectable at the cell population level. Each clone is represented by a pair of chromosomes (shown as vertical lines). 'X' — transcriptional silencing; arrows — expression (pink — maternal, blue — paternal). eQTL (expression quantitative trait locus) denotes effect of *cis*-regulatory allelic bias. **(b)** MAE and biallelic genes are interspersed throughout autosomes. Shown is partial allelic expression map of MAE genes (green triangles) and biallelic genes (yellow circles) on mouse chromosome 2 in mouse lymphoid cells (data from [8•]). **(c)** Combinatorial diversity arising from clone-specific MAE. The diversity within a normal cell population is reflected in the diversity of expression patterns from clone to clone. Note lack of coordination of allelic choice along the chromosome in the same clone, and independent choice of allelic state of the same gene between clones. Horizontal lines correspond to individual clones; triangles mark MAE genes on human chromosome 18, as measured in lymphoblastoid cells (data from [7]). At some loci, MAE is non-obligatory, in which case biallelic expression (yellow) is observed in certain clones. Unlike in parental imprinting, MAE can occur either from maternal (pink) or paternal (blue) allele. In some clones, expression is not determined. **(d)** Impact of MAE mapping resolution on the characterization of regulatory elements. If mapping resolution is low (top), candidate regions include both MAE and biallelic genes, which complicates the identification of common regulators of MAE such as hypothesized boundary elements. If mapping resolution is high (bottom), regulatory elements can be identified using a systematic approach (see text).

cell populations revealed that MAE affects about 10% of ~4000 tested genes in human lymphoblasts and about 15% of approximately 1300 assessed genes in equivalent mouse cells [7,8•]. Ribosomal DNA genes were also reported to be subject to monoallelic expression [12], as were certain neuronal genes [13,14]. Taken together, this adds up to between 10 and 20% of all mammalian genes, which is likely a significant underestimate, since tissue-specific MAE has not yet been systematically explored.

The genetic mechanisms that control establishment and maintenance of the widespread MAE remain to be discovered. However, currently available observations already allow us to put some limits on what kinds of mechanisms might be involved.

MAE genetic control hypothesis

We posit that autosomal clone-specific MAE is a regulated process that depends on specific sequences in the genome. The definitive evidence would involve identification of one or more of such sequences and experimental demonstration that they are necessary and sufficient to impart MAE in transgenic experiments. Such perfect proof is currently absent, and more data is needed to identify compelling candidate regulatory sequences for a typical MAE gene. Here we review observations that suggest the genetic control hypothesis.

Box 1 Functional properties of widespread clone-specific autosomal monoallelic expression

While MAE is obligatory for olfactory receptors, it is *non-obligatory* for a large majority of MAE genes: a given MAE gene can be biallelic in some clonal lineages (Figure 1a, gene *v*).
Biallelic expression corresponds to higher transcript level than monoallelic expression of the same gene in the next clone. Caveat: this is based on assessing a small number of genes [6,7].
Except for some special cases (olfactory receptors, ribosomal RNA genes), MAE genes do not generally cluster in the genome. They are *interspersed* throughout the genome, and are typically flanked by biallelic genes (Figure 1b).
Multiple genes are MAE in a given cell. Allelic choice for each MAE gene is apparently independent of choice at any other locus. There is no chromosome-wide coordination (unlike in X-inactivation). This results in potentially astronomical *combinatorial diversity* between clonal lineages of otherwise similar cells [7,8•] (Figure 1c).
Extreme allelic bias is typical for MAE (10-fold or more), though RNAseq-based approaches also reveal examples of more attenuated bias.
At least in mouse, some MAE genes show *skewed* choice, where one allele has a higher likelihood of being expressed than the other allele (Figure 1a, genes *vi* and *vii*).

To provide clues as to possible mechanisms, we will also briefly describe known regulatory features involved in initiation and maintenance of X-inactivation, imprinting, and MAE. Finally, we will sketch the lines of investigation that should allow identification of the hypothetical MAE regulatory elements.

Indications of sequence-dependence of MAE

A specific subset of genes are subject to MAE. Other genes have shown biallelic expression in all tested clones from all assessed individuals [7,8•]. While this observation was made on a modest number of clones assessed so far, it suggests that the propensity to be MAE or to be always biallelic is an intrinsic property of genes. This is further supported by the observation that MAE significantly overlaps within mouse and human orthologous genes [8•], consistent with the existence of conserved regulatory elements in close linkage with these genes. Perhaps most tellingly, a subset of MAE genes appears to show systematic bias in choosing which allele will be active in a given lineage. While current observations [8•] cannot exclude parent-of-origin effects, they strongly resemble the 'primary skewing' of X-inactivation due to *cis*-effect of particular variant of the *Xce* locus [15–18]. An example of such skewing is that in a cross between mice of Cast/Ei and 129 strains, only about 20% of cells in a female embryo will have X^{129} active, while the rest will have X^{Cast} active.

The neighborhoods of known MAE genes are significantly enriched with certain genomic elements, including recombination hotspots [19], ancient gene duplications [20], and lower CpG density at the transcription start sites [21]. While none of these features have very high predictive value, this kind of correlation analysis should become more informative as the number of known MAE genes increases.

Known genetic features involved in separate control of alleles

The interplay of genetic and epigenetic mechanisms controlling X-inactivation, imprinting, and the expression of olfactory receptor and immunoglobulin genes has been studied for decades. Thus, drawing parallels with known genetic mechanisms involved in these processes is likely to be helpful in the identification of genetic features that control clone-specific autosomal MAE. Mechanisms that directly rely on genomic elements are highlighted in Table 1. One common theme is the presence of boundary elements that separate chromosome domains under different modes of transcriptional control; such elements often include binding sites for CTCF protein [22–24], reviewed in [5,25]. Higher-order chromatin organization also often involves CTCF binding at defined sites [26•,27,28]. Another common theme is DNA methylation, which can induce or prevent the binding of DNA-binding proteins (reviewed in [5,29]). DNA methylation relies on the presence of CpG dinucleotides at the right density and localization in the genome sequence. Yet another theme involves long non-coding RNAs, which can recruit chromatin modifying factors, and whose deletion or insertion can cause large-scale chromatin reorganization (rev. in [30]).

We can roughly estimate the incidence of such genetic features, based on their expected function. For example, the fact that MAE genes are largely interspersed in the genome (Figure 1b) strongly implies the existence of multiple boundary elements separating MAE genes from neighboring biallelic loci. These elements probably share many properties with each other (though uniqueness of each is not absolutely impossible). A natural approach to finding such numerous needles in a haystack is signal averaging: mapping as many MAE regions as feasible and looking for common features in these high-resolution, 'minimal' regions (Figure 1d).

Similar reasoning applies to other types of locally acting *cis*-regulatory elements that distinguish genomic areas containing MAE genes from biallelic neighbors. For example, the hypothetical regulatory elements involved in the skewed choice of MAE alleles are likely to be as numerous as the MAE genes showing primary skewing (probably on the order of a hundred in a sufficiently distant mouse cross). Identification of these elements could be facilitated by the comparison of sequence differences between involved strains — either in F1 crosses, or in the parental strains.

Regulatory elements with long-range (or even chromosome-wide) effects are likely to be few in number and may be amenable to deletion analysis. Recent work relied

Table 1

Recurring themes in genetic elements affecting X-inactivation, imprinting and MAE.

Type of epigenetically controlled allele-specific expression	Regulatory elements primarily related to	
	. . .establishment	. . .maintenance
Imprinting	Imprinting Control Regions (ICRs) marked by CpG methylation in germline — rev. in [5].	ICRs recognized by *trans*-acting factors; Chromatin states regulated by CpG methylation, CTCF or long non-coding RNAs — rev. in [5]
X-chromosome inactivation	X-chromosome inactivation center controls counting, choice and initiation via ncRNAs and higher-order chromatin organization [32] Xce locus controls primary skewing — rev. in [50] Repeat-rich 'way stations' for spreading of silencing [51]	ncRNA *Xist* and CpG methylation regulate silencing chromatin marks — rev. in [32] Boundary or dispersed elements (CTCF, LINE) help isolate genes that escape X-inactivation [22–24]. Structure and activity of X chromosome reflected in higher-order chromatin organization [26•]
MAE		
Olfactory receptor genes	*Cis*-regulatory and possible *trans*-regulatory elements important for locus choice [27,52] Presumable locus control regions — marked early via different replication timing [53]	Higher-order organization and subnuclear localization disrupted by lamin B receptor [54•] Presumable boundary elements needed for local escape from global chromatin silencing of olfactory receptor genes [55]
Immunoglobulins	Higher-order chromatin organization regulated by CTCF — rev. in [25] Differential CpG methylation — rev. in [56] Early differential marking of alleles [57•]	Rearranged DNA — rev. in [56]
The rest of MAE	Presumed elements responsible for primary skewing [8•] Higher-order chromatin organization regulated by CTCF at protocadherin gene clusters — rev. in [58]	ncRNA gene ASAR6 controls asynchronous replication and MAE — rev. in [31] Presumed boundary elements separate each MAE locus from flanking biallelic regions

on a 'chromosome engineering' approach to identify ASAR6, a large noncoding RNA locus controlling MAE on human chromosome 6 (reviewed in [31]), which bears similarity to the noncoding RNA *XIST* on the X-chromosome [32]. Intriguingly, ASAR6 deletion causes activation of nearby silenced alleles [33••,34]. The existence of such element implies possible mechanistic analogies between processes on the X and on autosomes.

Finally, differentially methylated regions are commonly involved in the establishment of allele-specific expression. DNA methylation analyses have helped in identification of allele-specific regulation, including X-inactivation and imprinting [35–37]. Extending these approaches to analysis of clonal lineages might be informative.

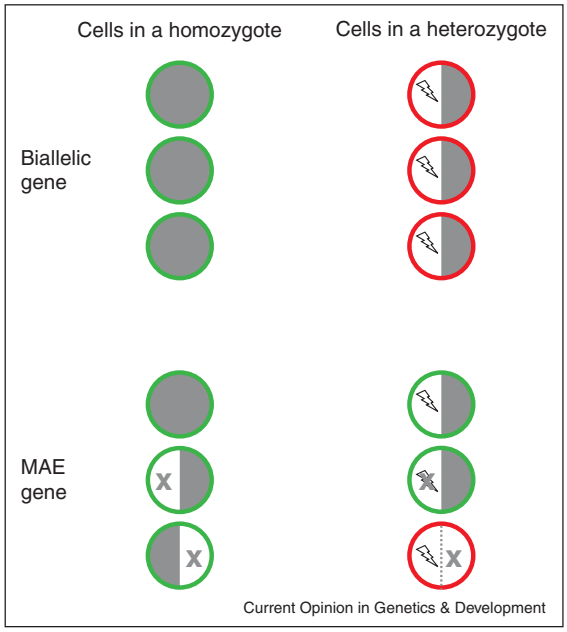
MAE, heterogeneity, and haploinsufficiency

In general, it is not clear what role MAE plays in the function of autosomal genes. For some genes, MAE may be adaptive. Allelic exclusion in the immunoglobulin genes is thought to confer unique antigen specificity to a given cell [38], while MAE of olfactory receptors is likely driven by the necessity of both ligand specificity and inter-cellular diversity [11]. For some MAE genes, such as interleukin-4, the difference between monoallelic and biallelic expression has been proposed as a mechanism of gene dosage regulation [39]. MAE might also be a side effect of some other feature of genome biology. Regardless of its adaptive role, MAE has distinct functional consequences for the affected genes.

The basis for these consequences is the epigenetic heterogeneity introduced by MAE. The clonal nature of MAE makes it a likely candidate mechanism for any number of mosaic effects. One intriguing possibility is the epigenetic clonal heterogeneity reportedly maintained in colon cancer and contributing to drug resistance in individual clones [40••]. Development of such mosaic drug resistance can be prevented by chromatin modifying agents [41], highlighting the potential of MAE-focused epigenetic treatments.

One intuitively clear consequence that MAE can have on the genotype–phenotype relationship is haploinsufficiency, whereby a diploid organism heterozygous for a loss-of-function allele shows a phenotype. On such a genetic background, epigenetically imposed monoallelic expression can cause complete loss of expression of the functional allele in a fraction of cells. This can lead to all-or-nothing differences in cellular phenotype. A striking example is the heterozygous deletion in the X-linked tumor suppressor *FOXP3* [42]. In heterozygous knockout mice, mammary tumors develop at a high rate. In heterozygous women, breast cancer nearly always arises from cells that have inactivated the X chromosome that includes the one functional copy of the gene, leaving

Figure 2



Haploinsufficiency in the context of biallelic expression and clone-specific monoallelic expression. If a haploinsufficient gene is biallelic, loss-of-function mutation (lightning symbol) leads to uniform halving of gene dose (gray area), and a uniform deficiency of function (red circle). In case of MAE gene, a large fraction of cells in the normal state (green circle) express half the dose (half-circle). When mutated allele is silenced, it is equivalent to cells having half the gene dosage; only when the mutated allele is the only one expressed (empty circle), cells show deficiency of function (red).

no expression of the functional copy. In other examples, remarkable functional differences have been observed as a result of autosomal MAE. The innate immunity receptor *Tlr4* is subject to MAE [43]. Mice heterozygous for a mutant variant of *Tlr4* have two kinds of lymphocytes: some (those expressing the functional copy) are responsive to lipopolysaccharides, while others (those expressing only the deficient allele) are not.

While haploinsufficiency is not necessarily linked to MAE, the resulting phenotype is likely to differ compared to haploinsufficiency of a biallelically expressed gene (Figure 2). In the case of tumor suppressor genes, partial haploinsufficiency (where genetic loss of the normal allele is required for disease progression [44]) is unlikely to involve MAE genes, since epigenetic silencing of that allele would by itself be sufficient for complete loss of function. In other cases, since cells containing MAE genes are heterogeneous with respect to gene dosage (Figure 2), haploinsufficiency is likely to result in variable penetrance. Variations in the eventual proportion of cells with normal dose of the gene (one active allele), compared to deficient cells (no active, functional alleles) can lead to a much broader range of phenotypes. In the case of X-inactivation, such secondary skewing (due to preferential survival of cells expressing a particular allele, or to random developmental bottlenecks) serves as an explanation for unusually varied phenotypes in carriers of *FMR1* gene mutations in Fragile X syndrome [45]. In Rett syndrome, another important X-linked neurodevelopmental disorder, some carriers of *MeCP2* mutant alleles have much milder phenotypes because of extreme skewing [46,47].

MAE-based haploinsufficiency, with its cell heterogeneity, functional sufficiency of one normal allele per cell, and the presence of the intact allele in each cell, should be amenable to a different set of therapeutic strategies than the uniform, biallelic type. Restoration of the activity of the epigenetically silenced allele is possible, at least in principle [48••]. Such reversal of the epigenetic allelic choice or de-repression of the silent allele (using genome-wide or locus-specific approaches) would essentially make the gene in question haplosufficient, since its tissue-wide function would be restored, even on heterozygous background. Together with taking into account particulars of the gene and mutation (see e.g. [49]), this is a promising goal for personalized medicine approaches.

Conclusion

MAE is a short-range autosomal analog of X-inactivation in that it creates epigenetic mosaicism: cells of the same type, in the same individual, have stable differences with respect to the set of expressed alleles. However, the number of affected genes and the diversity of this epigenetic mosaic are much greater. This transcriptional heterogeneity within cell types, in turn, creates nontrivial consequences at the systems level. Regulation of MAE appears as an intricate interaction of genetic and epigenetic mechanisms. The challenge resides not only in identifying mechanisms of MAE, but also in establishing their sequence over time and how they affect MAE function depending on the locus and developmental stage. Understanding of these mechanisms and the ability to manipulate them would open new possibilities for dealing with functional cell heterogeneity: from limiting drug resistance to correction of haploinsufficiency.

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