Mammalian *Su(var)* Genes in Chromatin Control

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Abstract

Genetic screens in *Drosophila* have been instrumental in distinguishing approximately 390 loci involved in position effect variegation and heterochromatin stabilization. Most of the identified genes [so-called *Su(var)* and *E(var)* genes] are also conserved in mammals, where more than 50 of their gene products are known to localize to constitutive heterochromatin. From these proteins, approximately 12 core heterochromatin components can be inferred. In addition, there are approximately 30 additional Su(var) and 10 E(var) factors that can, under distinct developmental options, interchange with constitutive heterochromatin and participate in the partitioning of the genome into repressed and active chromatin domains. A significant fraction of the Su(var) and E(var) factors are enzymes that respond to environmental and metabolic signals, thereby allowing both the variation and propagation of epigenetic states to a dynamic chromatin template. Moreover, the misregulation of human SU(VAR) and E(VAR) function can advance cancer and many other human diseases including more complex disorders. As such, mammalian *Su(var)* and *E(var)* genes and their products provide a rich source of novel targets for diagnosis of and pharmaceutical intervention in many human diseases.

Contents

INTRODUCTION

The information encoded by the DNA sequence determines the amino acid composition of proteins, provides binding sites for transcriptional regulators, influences nucleosome positioning, and instructs the higher order organization of chromosomes. Many chromatin components use various epigenetic mechanisms to interpret DNA-encoded information. Of the ~25,000 annotated mouse gene products, at least 10% are nuclear, and half of those show subnuclear localization (Dellaire et al. 2003, Sutherland et al. 2001). The number of mouse transcription factors (TFs) alone is estimated to be 1,600 (Kanamori et al. 2004).

To date, approximately 50 mammalian proteins have been shown to be enriched at one of the most prominent silenced chromosomal domains: pericentric, or constitutive, heterochromatin. Pericentric heterochromatin is largely devoid of genes, highly enriched for repetitive elements, and contributes to centromere function and chromosome segregation (Malik & Henikoff 2009, Peng & Karpen 2008). It seems paradoxical that such a large noncoding region also influences gene regulation and cell type differentiation. Although the total number of gene products discriminating heterochromatin versus euchromatin is currently not known, genetic screens for position effect variegation (PEV) in *Drosophila* indicate the involvement of ~150 loci for heterochromatin [so-called *Su*(*var*) genes] and ~240 for euchromatin [so-called *E*(*var*) genes] function (Eissenberg & Reuter 2009, Schotta et al. 2003).

Many, but not all, of the PEV modifiers discovered in *Drosophila* are conserved in other eukaryotes, such as *Saccharomyces cerevisiae* (Aparicio et al. 1991), *Schizosaccharomyces pombe* (Allshire et al. 1995, Grewal & Jia 2007), plants (Fischer et al. 2006, Henderson & Jacobsen 2007) and mammals, although their functions in these different model organisms have distinctive characteristics (for a general overview see Allis et al. 2007).

We have evaluated the literature over the past five years but also included earlier key studies, and with this review focus primarily on mammalian Su(var) and some E(var) factors. These components carry out diverse functions that not only are important for heterochromatin formation but are implicated in gene regulation, cell type identity and differentiation, genome stability, various diseases, and cancer. Importantly, they represent key components of chromatin biology that respond to external and metabolic signals that allow epigenetic modulation of the DNA/chromatin template under varying physiological and pathological conditions.

IDENTIFICATION OF *Su(var)* AND *E(var)* GENES IN *DROSOPHILA*

Position effects describe the variable expression of a DNA sequence, as the state of adjacent chromatin modifies it in a quantitative and/or temporal manner. These differences due to changes in chromatin state normally occur during development or are serendipitously exposed if a euchromatic DNA sequence is translocated into heterochromatin. A classical example of such a PEV is a radiation-induced genomic rearrangement in *Drosophila* that inserts the *white* gene into the vicinity of pericentric heterochromatin (Muller 1930). The *white* gene encodes for a red eye pigment in wild-type flies (the gene is named after the mutant white eye phenotype). Upon rearrangement, known as *white mottled 4h* (w^{m4h}), the *Drosophila* eye displays variegated red and white patches that are caused by random silencing of the *white* gene in clonal cells of the developing eye imaginal disc (**Figure 1*a***).

The w^{m4h} indicator strain allowed the development of genetic screens following chemical mutagenesis for chromatin regulators to identify mutations that alter the red/white pigment distribution (Donaldson et al. 2002, Eissenberg & Reuter 2009, Grigliatti 1991, Schotta et al. 2003). These PEV modifier screens distinguished two major classes of genes. The first class, called *Suppressors of variegation* or *Su(var)*, resulted in conversion to nearly full red eye pigmentation, as the w^{m4h} epiallele becomes stably activated in almost all cells of the developing eye imaginal disc. Therefore, *Su(var)* mutations weaken heterochromatin establishment and/or maintenance, consistent with the localization of most wild-type *Su(var)* gene

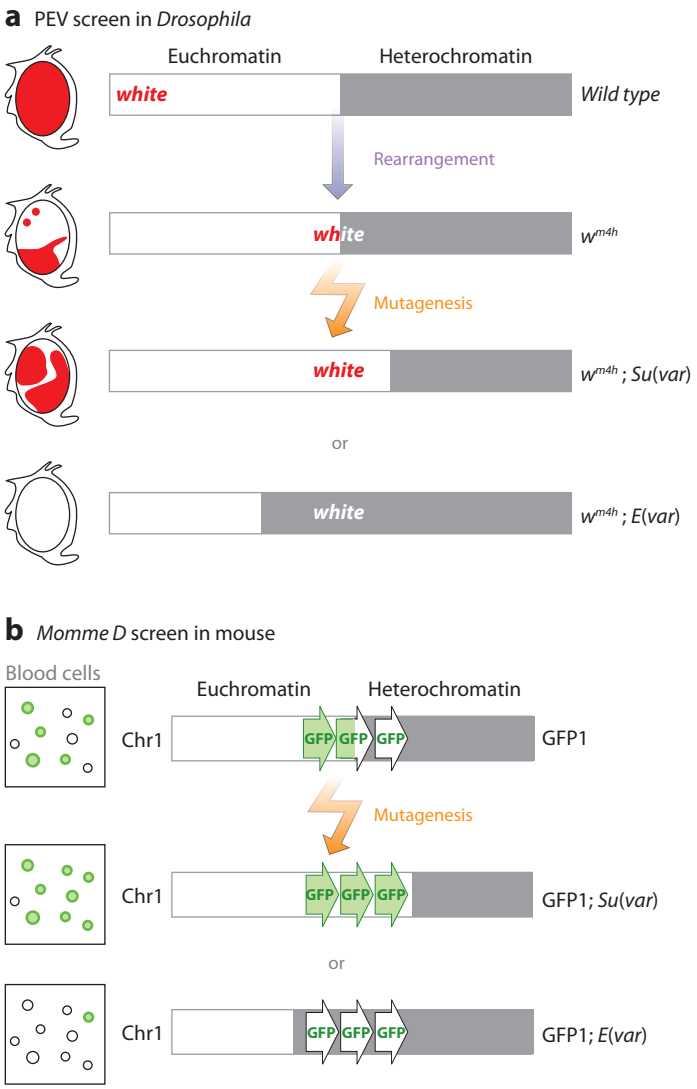

Figure 1

Outline of genetic screens used to identify dominant modifiers of position effect variegation (PEV) in *D. melanogaster* (*a*) and mouse (*b*). *Su(var)*, *Suppressors of variegation*; *E(var)*, *Enhancers of variegation*; *Momme D*, *modifiers of murine metastable epialleles*; Chr1, chromosome 1; GFP, green fluorescent protein.

products to heterochromatin (see below). The second class, called *Enhancers of variegation* or *E*(*var*), resulted in almost completely white fly eyes, indicating that the w^{m4h} epiallele can no longer be activated. Therefore, *E*(*var*) mutations either diminish euchromatin or allow the expansion of heterochromatin. Because most of the wild-type *E*(*var*) gene products do not accumulate at heterochromatin, their intrinsic function appears to be in euchromatin, with antagonizing effects on the stabilization of heterochromatin.

Su(*var*) and *E*(*var*) genes in *Drosophila* can be further refined by their involvement in at least five alternative silencing pathways that depend on the chromatin position of the PEV epiallele (Girton & Johansen 2008, Phalke et al. 2009). In addition to pericentric PEV, these include telomeric, chromosome 4, retrotransposon, and tandem repeat silencing. Although there is significant, but not full, overlap of *Su*(*var*) and *E*(*var*) gene function with these distinct PEV epialleles, the RNAi machinery appears to be primarily involved in retrotransposon and tandem-array repression (Pal-Bhadra et al. 2004), with only minor effects on pericentric PEV (G. Reuter, unpublished observations). The most robust function in PEV modification is provided by loss-of-function mutations that can be rescued by a wild-type genomic copy. For this review, we will primarily integrate *Drosophila* PEV modifiers of w^{m4h} or similar (e.g., *T*(*2;3*) *Stubble*^{v}) pericentric epialleles.

Approximately 150 independent *Su*(*var*) loci have been identified in *Drosophila*, of which approximately 10% were isolated by positional gene mapping. The catalog of molecularly characterized *Drosophila Su*(*var*) genes was expanded by chromatin regulators identified by gene-specific experimental approaches, rather than a PEV screen, and which were only later shown to modify PEV. Together, the molecular identities of approximately 60 *Su*(*var*) and 25 *E*(*var*) genes are known in *Drosophila* to date (see **Supplemental Tables 1** and **2**, follow the Supplemental Material link from the Annual Reviews home page at **http://www.annualreviews.org**).

Supplemental Material

IDENTIFICATION OF PEV MODIFIER GENES IN THE MOUSE

In mice, a genetic screen to identify dominant *modifiers of murine metastable epialleles* (*Momme D*) was performed, using a green fluorescent protein (GFP) transgene-array insertion in mouse chromosome 1 (Blewitt et al. 2005) (**Figure 1*b***). This GFP transgene array is intermediately expressed in red blood cells, where fluorescence-activated cell sorting (FACS) analysis indicates that ~55% of erythrocytes are GFP-positive. Following chemical mutagenesis, more than 2,500 offspring from an inbred F1 cross were screened to identify mice that had either an increased ratio [*Su*(*var*) phenotype activating the transgene array] or a reduced number [*E*(*var*) phenotype silencing the transgene array] of GFP-positive cells. From this screen, approximately 25 *Momme D* mutants have been identified (E. Whitelaw, personal communication), of which <10 have been isolated by positional gene mapping (Ashe et al. 2008, Blewitt et al. 2008). Momme D examples include DNA (Dnmt1, Dnmt3b)- and histone [histone deacetylase 1 (Hdac1)]-modifying enzymes, components of chromatin remodelers (Baz1b, Snf2h/Iswi), transcriptional corepressors (Trim28 or Tif1b/Kap1), and a factor for the structural maintenance of chromosomes (Smchd1) (E. Whitelaw, personal communication).

ACCUMULATION OF SU(VAR) PROTEINS AT MOUSE HETEROCHROMATIN

Immunofluorescence (IF) of *Su*(*var*) and *E*(*var*) gene products at polytene chromosomes in *Drosophila* larvae revealed chromocenter association for HP1 (James & Elgin 1986) and SU(VAR)3–7 (Cleard & Spierer 2001, Reuter et al. 1990) as well as a few other SU(VAR) proteins, but largely not for *E*(*var*) gene products. Similarly, many mammalian orthologs of *Drosophila Su*(*var*) gene products accumulate at pericentric heterochromatin, as detected by IF of endogenous proteins or after epitope tagging

(e.g., GFP). Mouse cells in particular contain easily detectable (in human cells this is much less pronounced) constitutive heterochromatin that is composed of large arrays (more than 10,000 copies of a 231 bp unit) of A/T-rich major satellite repeats in the pericentric regions of mouse chromosomes (Vissel & Choo 1989). These large pericentric domains differ from centric heterochromatin, which is important for kinetochore attachment and chromosome segregation (Allshire & Karpen 2008, Cleveland et al. 2003, Malik & Henikoff 2009, Peng & Karpen 2008) and can be readily visualized by 4′,6-diamidino-2-phenylindole (DAPI) staining, which reveals approximately 15–20 heterochromatic foci in interphase chromatin (**Figure 2**). Accumulation at these DAPI-dense foci is a robust indication that chromatin

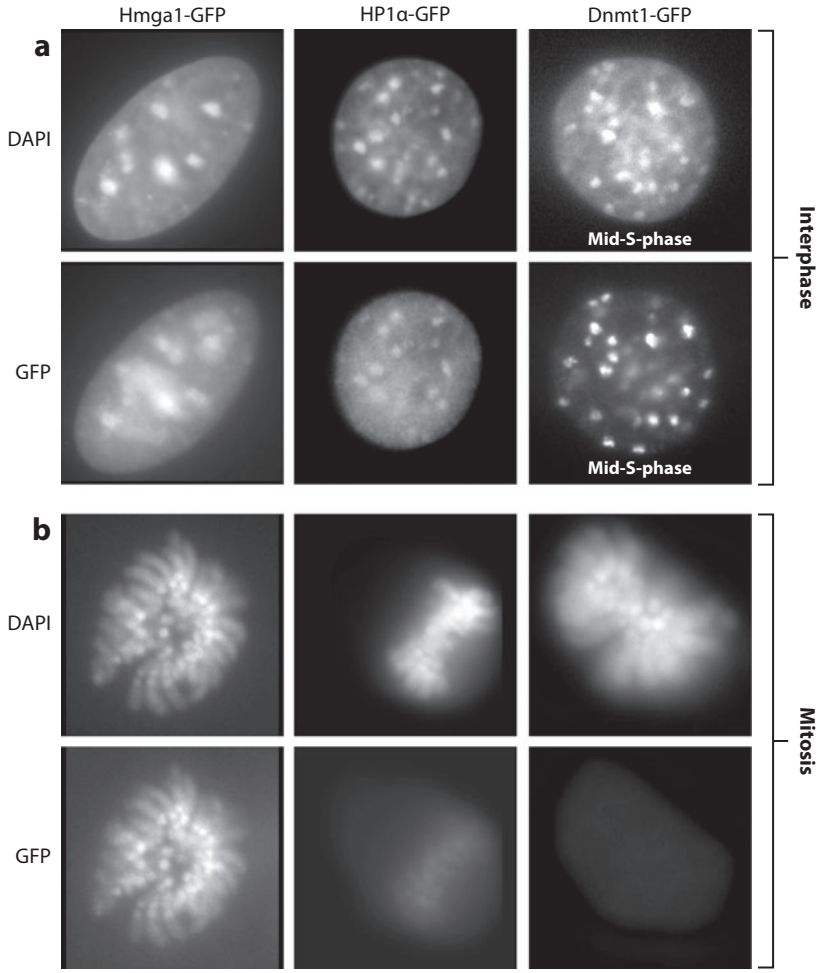

Figure 2

Identification of proteins localizing to pericentric heterochromatin in mouse fibroblasts. AT-rich pericentric repeats are revealed as heterochromatic foci after 4′,6-diamidino-2-phenylindole (DAPI) staining in interphase nuclei (*a*) and in mitotic chromosomes (*b*). Hmga1, HP1α, and Dnmt1 were GFP-tagged at their endogenous loci in NIH3T3 cells. Localization of Hmga1-GFP and HP1α-GFP overlaps with DAPI-dense foci in interphase cells, whereas Dnmt1-GFP accumulates around heterochromatin only in mid- to late S-phase. Mitotic chromosomes retain Hmga1-GFP, whereas most of HP1α-GFP and all of Dnmt1-GFP is dispersed.

factors directly contribute to heterochromatin structure.

We reviewed the described chromatin association of murine *Su(var)* and *E(var)* candidate products, including two published screens of subnuclear localization in mouse cells (Poser et al. 2008, Sutherland et al. 2001), and also performed a GFP-tagged, gene-trap approach to identify heterochromatin components in mouse fibroblastic cells (B. Fodor & T. Jenuwein, unpublished data). Among the identified gene products were Hmga1, Hmga2, and HP1α, whose colocalization in mouse fibroblasts with DAPI-dense foci during interphase and at mitotic chromosomes is shown in **Figure 2**. Also included is Dnmt1, which transiently associates with heterochromatin during DNA replication. This screen could not identify intronless genes, such as histone H1.

Combining the available IF data suggests that approximately 50 mouse proteins accumulate at pericentric heterochromatin, in either fibroblasts, early embryonic tissues, or B or T cells. Of those, 30 have orthologs in *Drosophila*. Taking both the genetic dissection of pericentric PEV modification in *Drosophila* and the accumulation at heterochromatin in mouse cells of orthologous gene products as stringent criteria, approximately 12 factors (highlighted in yellow in **Table 1**) appear to be core heterochromatin components in proliferating somatic cells. These are: histone H1 and the histone variant H2a/z, the chromatin-modifying enzymes Suv39h1 (KMT1a) and Suv39h2 (KMT1b), Suv4-20h1 (KMT5a) and Suv4-20h2 (KMT5b), Hdac2, the chromatin binders HP1α and HP1β, the high-mobility-group proteins Hmga1 and Hmga2, components of chromatin remodelers such as Atrx, the transcriptional corepressor Trim28 (Tif1b/Kap1), and DNA methyl-binding domain factors such as members of the Mbd family. These approximately 12 central heterochromatic modules appear to identify the basic composition of constitutive heterochromatin in both *Drosophila* and mammals.

ADDITIONAL MECHANISMS CONTRIBUTE TO CONSTITUTIVE HETEROCHROMATIN

Despite the stringent cross-species criteria, there are apparent differences between heterochromatic silencing processes in *Drosophila* and mammalian cells. This is most prominent for DNA methylation, which is sparse only in *Drosophila* (Lyko et al. 2006). Thus, the components needed for DNA methylation (Cedar & Bergman 2009, Goll & Bestor 2005, Suzuki & Bird 2008)—such as the DNA methyltransferases Dnmt1, Dnmt3a and Dnmt3b; the intermediary hemi-methyl 5-me CpG binding factor Uhrf1 [ubiquitin-like with plant homeo domain (PHD) and Ring finger], also called Np95; other 5-me CpG binders, such as Mecp2, Zbtb4, and Kaiso; and the remodeling component Lsh (lymphoid specific helicase), which functionally links histone and DNA methylation (De La Fuente et al. 2006, Yan et al. 2003a)—all represent intrinsic heterochromatin elements in mammals that cannot be examined for PEV modification in flies. By contrast, the contribution of RNAi components to mouse pericentric heterochromatin remains questionable (Kanellopoulou et al. 2005, Murchison et al. 2005), and Piwi-like RNAi modules appear to operate primarily during germ cell formation/meiosis (Brennecke et al. 2008), protect from transposon mobilization, and have clustered chromatin targets at intergenic regions but not at pericentric heterochromatin (Girard & Hannon 2008). However, cell cycle–regulated transcription of major satellite repeats (Lu & Gilbert 2007) and heterochromatic H3K9me3 marks are sensitive to RNAse treatment that removes dsRNAs (Maison et al. 2002), which suggest that heterochromatic transcripts could be potential structural components of heterochromatin.

There are also significant differences between the DNA sequences for mouse major satellite repeats (and in particular for human pericentric repeats that differ among individual chromosomes) and the heterochromatic

Table 1 Core Su(var) components as qualified by PEV modification in *Drosophila* and pericentric accumulation in mouse chromatin

Function	Mouse (protein)	*Drosophila* (*gene*)	Reference (mouse protein)
Histone variants	H1.0	***H1***	Catez et al. (2002)
	H2a/z[a]	***His2Av***	Bulynko et al. (2006)
Chromatin-modifying enzymes	Suv39h1 (KMT1A)	***Su(var)3-9***	Aagaard et al. (1999)
	Suv39h2 (KMT1B)		
	Suv4-20h1 (KMT5B)	***Suv4-20***	Schotta et al. (2004)
	Suv4-20h2 (KMT5C)		
	Hdac2[b]	***HDACs***	Rountree et al. (2000)
	AurkB	*Ial*	Crosio et al. (2002)
	Trim28 (Tif1b, MommeD9)	(***bonus***)	Ryan et al. (1999)
Chromatin binders	Hp1α, Hp1β	***Su(var)2-5*** (*HP1*)	Horsley et al. (1996), Wreggett et al. (1994)
	Hmga1, Hmga2	***D1***	Harrer et al. (2004), Sutherland et al. (2001)
	Cbx2[c]	*Pc*	Puschendorf et al. (2008)
	Rnf2[c]	*Sce*	Puschendorf et al. (2008)
	Bmi1[c]	*Psc*	Puschendorf et al. (2008)
	Phc2[c]	*ph-d*, *ph-p*	Puschendorf et al. (2008)
	Daxx	*DLP*	Ishov et al. (2004)
	Ssrp1	*Ssrp*	Ishov et al. (2004)
Nucleosome remodeling	Atrx	***dAtrx*** (*XNP*)	McDowell et al. (1999)
	Baz1a (Acf1, Wcrf180)**	***Acf1***	Sutherland et al. (2001)
	Baz1b (Wstf, MommeD10)**	***Acf1***	Bozhenok et al. (2002)
	Arp6	*Actr13E*	Ohfuchi et al. (2006)
	Lsh (Hells)*	–	Yan et al. (2003b)
DNA binders	Sall1, -3, -4	*salm*, *salr*	Netzer et al. (2001), Yamashita et al. (2007)
	C/ebpα, C/ebpβ	*slbo*	Berberich-Siebelt et al. (2006), Liu et al. (2007)
	Zfp57 (Kraz1)*	–	Matsuda et al. (2001)
	Zfp68 (Kraz2)*	–	Matsuda et al. (2001)
	Zfp97 (AI046551)*,[d]	–	Sutherland et al. (2001)
	Yy1	*pho*	Shestakova et al. (2004)
	Gfi1b[e]	*sens*, *sens-2*	Vassen et al. (2006)
	Ikaros[e]	–	Vassen et al. (2006)
	Helios[f]	–	Hahm et al. (1998)
DNA methylation	Dnmt3a,* Dnmt3b (MommeD14)*,[d]	–	Bachman et al. (2001)
	Dnmt1 (MommeD2)**	–	Rountree et al. (2000)
	Uhrf-1	–	Bostick et al. (2007), Papait et al. (2007), Sharif et al. (2007)
	Dmap1[b]	*DMAP1*	Rountree et al. (2000)
	Mbd1, -2, -4[d]	***MBD-like***	Hendrich & Bird (1998)
	Mecp2*	–	Brero et al. (2005)
	Zbtb4*	–	Filion et al. (2006)
	Zbtb38 (Cibz)*	–	Sasai et al. (2005)
	Kaiso*	–	Filion et al. (2006)
	Mbd3l1[b]	–	C.L. Jiang et al. (2004)

(*Continued*)

Table 1 (*Continued*)

Function	Mouse (protein)	*Drosophila* (*gene*)	Reference (mouse protein)
Chromatin replication	Orc1	*Orc1*	Lidonnici et al. (2004)
	Orc2l	*Orc2*	Auth et al. (2006)
	Pcna**	***mus209***	Takasaki et al. (1981)
	Cdc6	*Cdc6*	Auth et al. (2006)
Chromosome segregation	Incenp	*Incenp*	Parra et al. (2003)

Dominant PEV modifier genes for w^{m4b} or similar pericentric epialleles (e.g., *T(2;3) stubble*^{v}, *BL2*, and *Dp(1;f)γ878*) in *Drosophila* are shown in bold and are classified as *Su(var)* genes. Although the *bonus* mutant is an *E(var)* gene with w^{m4b}, it also displays a *Su(var)* phenotype with the *Dp(1;f)γ878* pericentric rearrangements. PEV modification has not been described for the other (not bold) *Drosophila* genes. All of the listed proteins have been reported to be enriched at mammalian pericentric heterochromatin. Highlighted in yellow are mouse proteins that, based on their pericentric accumulation and PEV modification of their *Drosophila* orthologous genes, have been qualified as core heterochromatin components, as shown in **Figure 3**. For a complete listing of *Drosophila Su(var)* and *E(var)* genes and their mouse orthologs refer to **Supplementary Tables 1** and **2** (follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**).

*Mouse proteins for which there is no *Drosophila* ortholog.

**Mouse proteins for which there is a strong PEV modifier ortholog but which only transiently associate with pericentric heterochromatin.

[a]In extraembryonic tissues.

[b]Shown in Chinese hamster ovary or other cells.

[c]In paternal pronucleus.

[d]Ectopic expression.

[e]In embryonic stem cells.

[f]B and T lymphocytes.

Supplemental Material

sequences at the *Drosophila* chromocenter (Peng & Karpen 2008). In addition, heterochromatic repeats and pericentric sequences are subject to evolutionary drift and allow considerable sequence variation (Malik & Henikoff 2009, Peng & Karpen 2008). Despite this, TF binding sites are embedded in the DNA sequences of various heterochromatic repeat sequences across diverse species. This can explain binding of the SU(VAR)3-7 zinc-finger factor to the *Drosophila* chromocenter (Cleard et al. 1997) as well as binding of the zinc-finger TFs Gfi1b (Vassen et al. 2006) and members of the Sall (orthologs of *Drosophila spalt*) family (Yamashita et al. 2007) to mouse pericentric heterochromatin. It is not unconceivable that the number of mammalian zinc-finger genes has evolved to >560 (Kanamori et al. 2004) to compensate for the diverse sequence variations in mouse and human heterochromatin. Thus, in addition to interaction with Trim28 (Zeng et al. 2008), pericentric association of Zfp57, Zfp68, and Zfp97 could, at least in part, also be directed by binding sites within heterochromatic repeat sequences.

THE BASIC UNIT OF CONSTITUTIVE HETEROCHROMATIN

Although the following does not necessarily reflect a sequential order, we will now discuss possible mechanisms for how core heterochromatin factors may synergize to establish and maintain interphase heterochromatin in proliferating cells. (*a*) The initial signal is probably binding sites that are embedded within the DNA repeat sequences for zinc-finger proteins (e.g., Gfi1b or Sall1) or other TFs. (*b*) Enrichment for histone variants, such as histone H1 and H2a/z, occurs. The pericentric H2a/z association needs further investigation, as H2a/z also functions to distinguish active promoters (Jin et al. 2009). (*c*) The high-mobility-group proteins Hmga1 and Hmga2 accumulate. These architectural proteins bind A/T-rich sequences and compete with histone H1 for nucleosome attachment. (*d*) Transcriptional corepressors, primarily Trim28, are recruited. Trim28 (a tripartite motif protein) is a sumo E3 ligase with a B-box type zinc-finger,

a bromo-, a PHD-finger, and a RING-finger domain. Autosumoylation is important for the repressive function of Trim28 (Zeng et al. 2008) and could explain why *bonus*, the *Drosophila Trim28* ortholog, in the context of Y heterochromatin can support both an *E*(*var*) and a *Su*(*var*) phenotype (Beckstead et al. 2005). (*e*) Atrx (α-thalassemia/mental retardation syndrome X-linked) is a member of the DEAD-like helicase superfamily that participates in ATP-dependent chromatin remodeling complexes. Although they serve many functions (Clapier & Cairns 2009), one of their roles in heterochromatin may be to facilitate replacement of histones that carry activating marks and/or to exchange histone variants (Goldberg et al. 2010). (*f*) The histone deacetylase Hdac2 and probably similar Hdac members are required to erase histone acetylation in preparation for the subsequent methylation of H3K9. (*g*) The lysine methyltransferases (KMTs) Suv39h1 (and Suv39h2) and their associated H3K9me3 binders HP1α and HP1β act to propagate heterochromatic H3K9me3 marks. (*h*) The KMTs Suv4-20h1 (and Suv4-20h2) synergize with the Suv39h-HP1 system to induce heterochromatic H4K20me3 marks. (*i*) Members of DNA methyl-binding domain (MBD) proteins, primarily Mbd1–4, bind DNA. Although not a function for *Drosophila* MBD-like, mouse Mbd4 (but not Mbd1–3) is also a DNA glycosylase, which has been shown to excise 5-me CpG, thereby actively removing DNA methylation in a DNA repair-coupled mechanism (Hendrich et al. 1999). (*j*) The full system for DNA methylation operates, as described above.

The molecular composition of this basic unit for mouse constitutive heterochromatin is shown schematically in the center diagram of **Figure 3**. An important hallmark is the vastly reiterative nature of this basic unit, which can amplify pericentric accumulation of the core heterochromatin components up to 1,000-fold. Moreover, many enzymes (deacetylases, DNA and histone methyltransferases, E3 ligases, helicases, ATPases) and transcriptional output converge at this heterochromatin structure. Thus, despite its apparently rather stable (but not entirely silent) propagation during cell divisions, constitutive heterochromatin represents a large platform for highly dynamic chromatin alterations.

During mid– to late–S phase of the cell cycle, several Su(var) factors transiently accumulate at pericentric heterochromatin. Among these are components of the basic replication machinery, such as the proliferating cell nuclear antigen (Pcna), which provides a central interphase to incorporate newly synthesized histones and recruit other chromatin-modifying components (Dnmt1, Hdac2, Pr-Set7, Asf1 and Baz1b) that appear to be necessary for epigenetic inheritance, as reviewed elsewhere (Campos & Reinberg 2009, Probst et al. 2009). Other replication-coupled components, such as Orc1 and Orc2, are not further detailed here, nor are factors involved in mitosis and chromosome segregation, such as Pr-Set7, AurB, Incenp, Wapl, and mCaps (Belmont 2006, Campos & Reinberg 2009, Hudson et al. 2009, Probst et al. 2009).

WHAT DISCRIMINATES HETEROCHROMATIN FROM EUCHROMATIN?

The same basic mechanisms, such as DNA sequence information, TF binding, transcriptional corepressors, chromatin remodelers, histone variants, chromatin-modifying enzymes and histone and DNA modifications, operate in both heterochromatin and euchromatin and are presented here with their *Su*(*var*) and *E*(*var*) gene functions (see **Table 1** and **Supplemental Tables 1** and **2**, follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**). There appears to be no "magic" heterochromatin component that would exclusively identify heterochromatin. How is heterochromatin then discriminated from euchromatin? The attributes needed for the establishment of heterochromatin probably follow three basic principles: (*a*) a repeat-rich DNA sequence that is largely devoid of fully functional promoter and enhancer signatures; (*b*) the higher

Supplemental Material

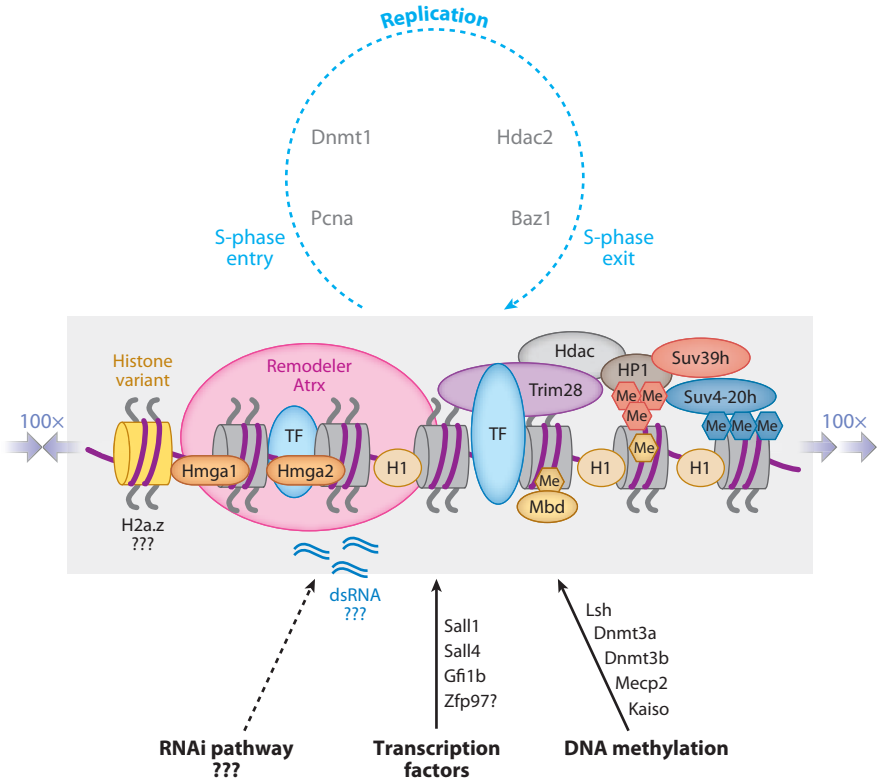

Figure 3

Core components of mouse pericentric heterochromatin in proliferating somatic cells. The indicated factors were determined to be core heterochromatin components on the basis of their pericentric enrichment in mouse chromatin and the strong PEV modification of their orthologous genes in *Drosophila* (see **Table 1**). Shown is a hypothetical repeat unit that can be reiterated up to 1000 times. Several transcription factors (*middle arrow*) that may recognize DNA sequence motifs within major satellite repeats as well as key modules for DNA methylation (*right arrow*) are also listed. During replication, additional components become transiently enriched at pericentric heterochromatin. A functional link between the RNAi machinery and pericentric heterochromatin still needs to be determined in mammals. TF, transcription factor; Me, methyl.

probability of generating aberrant RNA transcripts, such as unprocessed RNAs or dsRNA, from this repeat-rich DNA template (Zaratiegui et al. 2007); and (*c*) the mere size of the repeated DNA arrays, which can be composed of up to several Mbp in mouse pericentric heterochromatin. It seems that these qualities of the underlying DNA sequence would be sufficient first to select the appropriate components from the pool of the Su(var) and E(var) factors while scrutinizing RNA output and then to suppress an excess of nonproductive and/or aberrant RNA transcripts. Once established over such a large DNA region and reinforced by the reiterative nature of the DNA repeat units, heterochromatin will become a stable, self-amplifying and self-propagating entity over many cell generations. The notion of the self-propagation of larger chromosomal domains has been explained for similar contexts that are directed by the DNA double helix (Misteli 2001, Misteli 2007) and is consistent with recent evidence for the stable epigenetic inheritance of chromatin states by replication timing (Lande-Diner et al. 2009, Jorgensen et al. 2007) and for the altered topological

organization of heterotypic nucleosomes at centromeric regions (Cleveland et al. 2003, Furuyama & Henikoff 2009) as well as for triple helical structures that have been detected at *Drosophila* (Horn et al. 2004) and mouse (Lee et al. 1987) heterochromatin.

SU(VAR) AND E(VAR) FUNCTION OUTSIDE CONSTITUTIVE HETEROCHROMATIN

Because *Su*(*var*) and *E*(*var*) gene products represent basic components of overall chromatin biology, they are also functional outside the large heterochromatic landscape. Several of the core heterochromatin elements (**Figure 4**, *middle circle*) significantly exchange with telomeres (Schoeftner & Blasco 2009), imprinted regions (Feil 2009), senescence-associated foci (Krizhanovsky et al. 2008, Schmitt 2007), and rDNA regulation in the nucleolus (McStay & Grummt 2008).

Conversely, 43 additional Su(var) and 19 other E(var) factors (see **Supplemental Tables 1** and **2**, follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**) display a rather uniform subnuclear localization. Although these modules could also transiently interchange with constitutive heterochromatin, their chief activities reside at other chromatin regions, where they are relevant for gene regulation, cell type specification, RNA processing, chromosome segregation, and other functions (**Figure 4**).

Most influential among these additional Su(var) and E(var) factors are members of the Polycomb (Pc-G) and trithorax (trx-G) groups, and include Ezh2, Epc, and Suz12 [Su(var) factors] as well as Mll, Ash1l, and Jarid1 [E(var) factors]. Although some of the mouse Pc-G proteins associate with constitutive heterochromatin immediately after fertilization and prior to blastocyst formation (Puschendorf et al. 2008), they have significant roles at the inactive X chromosome (Xi) (Chow & Heard 2009, Senner & Brockdorff 2009). Moreover, it is known that the Ezh2-Pc system can partially

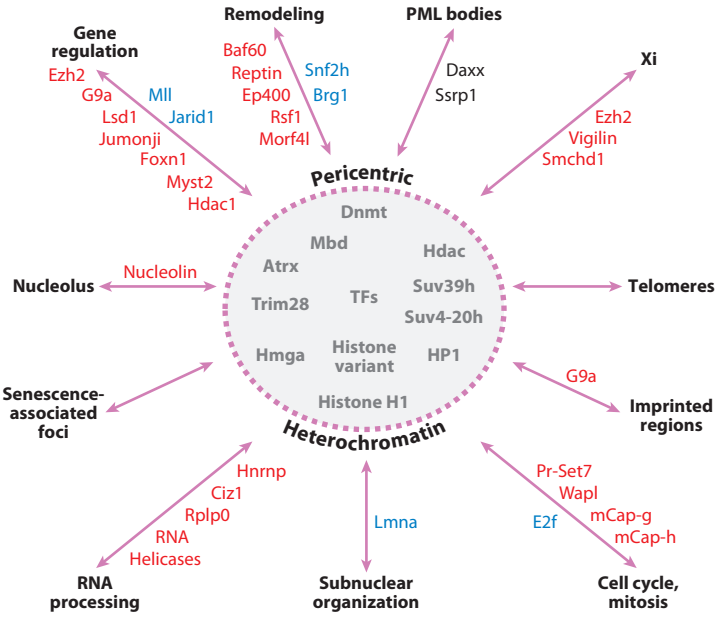

Figure 4

Interchange of mouse Su(var) and E(var) factors between subnuclear compartments. Core heterochromatin factors (in the central oval) also function outside pericentric heterochromatin at various subnuclear compartments and in many chromatin-dependent cellular processes. Exchange of core heterochromatin components with the subnuclear compartments is not further indicated. In addition, other mouse Su(var) (*red*) and E(var) (*blue*) factors from **Supplemental Tables 1** and **2** (follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**) are displayed. Their specific involvement in regulating chromatin structure at distinct subnuclear compartments is highlighted adjacent to their respective double-headed arrows, which reflect possible interchange with pericentric heterochromatin. Daxx and Ssrp1 are shown in black because PEV modification of their orthologous *Drosophila* genes has not been described. Xi, inactive X chromosome; PML, promyelocytic leukemia.

compensate for the Suv39h-HP1 pathway to protect pericentric heterochromatin (Peters et al. 2003, Puschendorf et al. 2008). Ezh2 is the major repressing H3K27me3 KMT that is antagonized by Mll (mixed lineage leukemia), a key activating H3K4me3 KMT; together they control the chromatin state of developmentally regulated promoters via the establishment of bivalent (H3K27me3/H3K4me3) histone marks (Azuara et al. 2006, Bernstein et al. 2006). The central functions of Pc-G and trx-G in stabilizing cell type specification and in protecting stem cell identity have been well documented (Bracken & Helin 2009, Hansen et al. 2008,

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Pietersen & van Lohuizen 2008, Ringrose & Paro 2007, Schwartz & Pirrotta 2007).

Gene repression at euchromatic positions is mediated by the Su(var) factors G9a (H3K9me2 KMT1c), Lsd1 [H3K4me3 lysine demethylase 1 (KDM1)], Jumonji (so far no identified activity), and several HDACs, e.g., Hbo1/Myst2 and Hdac1, and is further coregulated by Trim28 and Jarid1 (H3K4me3 KDM5), both of which can display dual activities as Su(var)/E(var) factors. Chromatin remodeling also extensively affects gene regulation through the repressive Su(var) factors Baf60 (Smarcd1), Ep400, reptin (Ruvbl2), Rsf1, and Morf4l and can be antagonized by the positively acting components Snf2h/Iswi (Smarca5) and Brg1 (Smarca4). Smarc defines SWI/SNF-related, matrix-associated, actin-dependent regulators of chromatin of the DNA and RNA helicase superfamilies with DExD and SANT domains that can mobilize nucleosomes (Cairns 2007, Clapier & Cairns 2009, Narlikar et al. 2002).

Intriguingly, the more accessible chromatin state observed in mouse embryonic stem (ES) cells (Meshorer et al. 2006) appears to be maintained by the increased activity of chromatin remodelers, including several E(var)-related factors (e.g., Brg1 and Chd1) (Fazzio et al. 2008, Gaspar-Maia et al. 2009). During differentiation, ES cell chromatin apparently becomes more restricted, in part through the establishment of large, cell type–specific H3K9me2-decorated chromatin domains that depend on the G9a and Ehmt1/Glp KMTs (Wen et al. 2009). These chromatin alterations may also be important for cellular reprogramming (Takahashi & Yamanaka 2006), as inhibition of G9a by a small molecule inhibitor (BIX-01294) facilitates cell fate transitions (Shi et al. 2008).

Other compartments that are connected to pericentric heterochromatin include promyelocytic leukemia (PML) bodies via the dual localization of Atrx and Daxx (Death-associated protein) in a cell cycle–dependent manner (Ishov et al. 2004). These two factors are also involved in the deposition of the histone H3.3 variant at telomeres (Goldberg et al. 2010). Cell cycle regulation of pericentric heterochromatin during mitosis involves the E(var) TFs of the E2f family and the Su(var) factors Pr-Set7, Wapl, and mCaps. Whether any of the Su(var) factors for RNA processing (A/B-type Hnrnps, Ciz1, nucleolin, Rplp0, and RNA helicases) participate in the regulation of mouse major satellite transcripts is currently unresolved. Finally, perturbed subnuclear organization, as exemplified by lamin A (Lmna) [E(var) factor] mutations in progeria and other syndromes (Dechat et al. 2008) significantly dysregulates heterochromatin function.

It is likely that the considerable exchange of Su(var) and E(var) factors between individual subnuclear compartments and the large heterochromatic platform greatly varies in distinct cell types and during different proliferative states. In addition, there could also be significant cross-participation among the diverse chromatin sections. The organization of chromosome territories is an important mechanism to canalize gene expression programs and assimilate subnuclear functions, as has been reviewed elsewhere (Fraser & Bickmore 2007, Kumaran et al. 2008, Lanctot et al. 2007).

SIGNALING PATHWAYS DIRECT SU(VAR) AND E(VAR) FACTORS TO CHROMATIN TARGETS

Approximately 8–10 major signaling pathways in eukaryotic cells are universally used to govern competence and response to changing external and intrinsic signals. We have cross-referenced the publicly available databases (Pathway Interaction Database and PubMed) with the identified mammalian (both mouse and human) Su(var) and E(var) factors from **Table 1** and **Supplemental Tables 1** and **2** (follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**) to reveal their described roles in diverse signaling pathways. This comparison indicates involvement for many Su(var) and E(var) factors in these pathways (**Table 2**). Although it is beyond the scope of this review to detail each of

Table 2 Mammalian Su(var) and E(var) factors linked to signaling pathways

Signaling pathway	Su(var) and E(var) factors
Steroid hormones/nuclear receptors	Hdac1, Hdac2, Hdac3; Pias1, -2, -3, -4; Baz1a (Acf1, Wcrf180), Baz1b (Wstf); Trim24 (Tif1a), Trim28 (Tif1b); C/Ebpα; Smarca4 (Brg1); Smarcd1 (Baf60a), Smarcd3 (Baf60c); Sirt1; Dnmt1; Dnmt3a, Dnmt3b; Serbp1; Ezh2 (KMT6); Ciz1; G9a (KMT1c); Lsd1 (KDM1a); Hbo1/Myst2; Cdc6; Tada3l; Jarid1a (KDM5a)
TGF-β/BMP/Smad	Suv39h1 (KMT1a); Hdac1, Hdac2; C/Ebpα, C/Ebpβ; Smarca4 (Brg1); Pias1, -2, -3, -4; Foxn1; Serbp1; Ezh2 (KMT6); Yy1
G protein–coupled receptors and **receptor tyrosine kinases** (ERK/MAPK, PI3K/AKT, JAK/STAT, JNK, PKC)	Hmga1; Trim28 (Tif1b); C/Ebpα, C/Ebpβ; Smarca4 (Brg1); Pias1, -2, -3, -4; Foxn1; Mbd4; Lmna; Mll1 (KMT2a), Mll2 (KMT2b); Ezh2 (KMT6); Bmi1; Nucleolin; Rps6ka5
WNT/β-catenin	Hdac1, Hdac2; Sall1, Sall4; Mbd2; Kaiso; Dnmt3a, Dnmt3b; Smarca4 (Brg1); Ezh2 (KMT6); Foxn1; Tada3l; Pontin (Ruvbl1), Reptin (Ruvbl2); E2f; Suz12
Notch/Delta	Sirt1; Smarcd3 (Baf60c); Foxn1; Yy1; Ikaros; Asf1
Hedgehog	Hdac1, Hdac2; Sall1, Sall3; Pias1; Mll1 (KMT2a), Mll2 (KMT2b); Epc1
TNF-α/NF-κB	Hdac3; Pias1, -2, -3, -4; Sirt1, -6; G9a (KMT1c); Yy1; Rsf1

Shown are mammalian Su(var) and E(var) factors involved in signaling pathways. The Su(var) and E(var) factors are from the core components in **Table 1** and include additional modifiers as listed in **Supplementary Tables 1** and **2** (follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**). Both human and mouse Su(var) and E(var) factors were referenced against the Pathway Interaction Database (**http://pid.nci.nih.gov**) (Schaefer et al. 2009) and PubMed (**http://www.ncbi.nlm.nih.gov**), but for simplicity only the mouse nomenclature is shown.

these connections, we will give some key examples that highlight important functions (and complexities) for Su(var) and E(var) factors in signal transduction to distinct chromatin targets (**Figure 5**).

In hormone signaling, the androgen receptor (AR), a subclass of the steroid hormone/nuclear receptor family, interacts, in a ligand-dependent manner [e.g., after binding to dihydrotestosterone (DHT)], with the protein-kinase-C-related kinase 1 (PRK1) that phosphorylates histone H3T11 to induce demethylation of H3K9me3 via the cooperative activities of the JMJD2C and LSD1 KDMs (Metzger et al. 2008, Wissmann et al. 2007). This combined signaling leads to activation of the PSA (prostate specific antigen) promoter. The involvement of LSD1 is puzzling because this Su(var) enzyme should remove activating chromatin marks, as it is indeed described as H3K4me2 KDM (Shi et al. 2004). H3T11phos and/or the interaction with the other AR complex members may adjust the substrate specificity and neutralize the Su(var) function of LSD1. Intriguingly, another protein kinase (protein kinase C β I) has recently been shown to complex with the AR and to phosphorylate H3T6, thereby occluding the natural target site (H3K4me2) for LSD1 activity (Metzger et al. 2010). Similar context-dependent adaptations of substrate specificities have also become apparent for other members (e.g., several PHF enzymes) of the jumonji class of KDMs, paticularly if they are composed of multiple domains including PHD fingers (Feng et al. 2010, Horton et al. 2010). These or comparable biochemical modifications could, at least in part, explain why the gene products of certain *E(var)* genes (e.g., Rpd3/Hdac1, Bonus/Trim28 and Lid/Jarid1) can also promote repressive (Beckstead et al. 2005, Lloret-Llinares et al. 2008, Mottus et al. 2000) rather than activating functions.

Smads are intracellular transducers of diverse functions that translocate into the nucleus following Tgf-β (transforming growth factor β)-mediated phosphorylation to stimulate chromatin-dependent gene activation. Tgf-β-dependent interactions of Smad2/3/4 with Smarca4 (Brg1, an ATPase component of

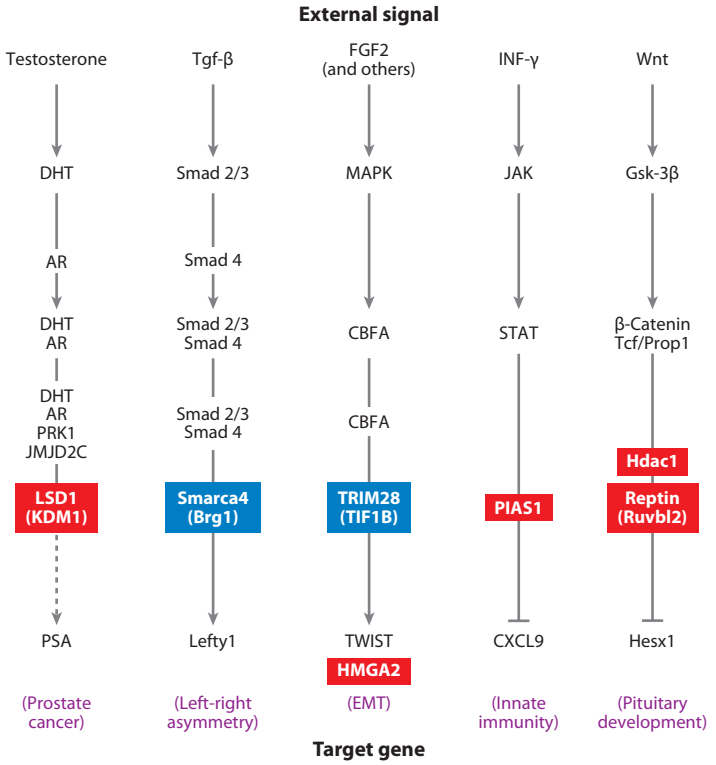

Figure 5

Examples of Su(var) and E(var) factors involved in signaling pathways. Stimulation with an external signal triggers a cascade that, through interactions of Su(var) (*red*) or E(var) (*blue*) factors, results in either activation (*arrows*) or repression (*inhibition lines*) of target genes. In the context of androgen receptor (AR)-mediated gene activation, the SU(VAR) factor LSD1 can confer a stimulating rather than a repressing function, as indicated by the dashed arrow.

chromatin remodelers) have been shown to activate several target genes that are important for instructing left-right asymmetry (Ross et al. 2006).

Receptor tyrosine kinases (RTKs) initiate signaling cascades that are amplified by ERK/MAP-K, PI3K/AKT, JAK/STAT, JNK, PKC, and other second-tier kinases. During epithelial-mesenchymal transition (EMT), fibroblast growth factor (FGF2), among other external signals, can trigger recruitment of important EMT mediators to target promoters for a subsequent transcriptional program that shifts epithelial to mesenchymal gene activity. Intriguingly, TRIM28 [an E(var)/Su(var) factor and E3 ligase] and HMGA2 [a Su(var) factor] are part of these EMT transcriptional cascades (Venkov et al. 2007). Another class of membrane-bound RTKs is cytokine receptors, which, for example, connect JAK/STAT signaling with Protein Inhibitors of Activated STAT 1 (PIAS1) [a Su(var) enzyme] in macrophages to repress a subset of interferon-γ (IFN-γ)-inducible genes by obstructing the recruitment of STAT to target promoters (Liu et al. 2004). The PIAS family is composed of common protein inhibitors of activated STAT that contain an E3 ligase with sumolyating activity and RING/zinc-finger domains.

Wnt/β-catenin signaling regulates functionally distinct transcriptional programs during development and disease. In addition to the canonical Lef/Tcf interaction, β-catenin can also complex with the homeodomain TF Prop1 during pituitary development. This leads to transcriptional repression of a lineage-inhibiting factor (Hesx1) through recruitment of reptin (RuvbL2) [a Su(var) factor and ATPase] and Hdac1 corepressors (Olson et al. 2006). Reptin (RuvbL2) and related proteins are AAA+ (ATPase associated with diverse cellular activities) ATPases (Jha & Dutta 2009) that are part of several nucleoprotein and chromatin remodeling complexes, some of which include other Su(var) and E(var) factors such as Morf4l, Rsf1, Ep400, and Baf60 (Smarcd1) (Cairns 2007, Clapier & Cairns 2009). Depending on their composition, these large protein complexes can either synergize or antagonize with other activating chromatin remodeling machines. Such an antagonism between a repressive human β-CATENIN-REPTIN-HDAC1 complex with a TIP60-HAT coactivator machine appears important to regulate transcription of a tumor suppressor gene (*KAI1*) that protects against metastasis (Kim et al. 2005). The REPTIN-HDAC1 interaction is stabilized by sumoylation of REPTIN (Kim et al. 2006), although it is not known whether this is mediated by one of the PIAS enzymes.

Although at a reduced incidence (see **Table 2**), Su(var) and E(var) factors have also been connected to Notch/Delta signaling,

Hedgehog response, and tumor necrosis factor-α (TNF-α)/NF-κB signals. These cases include Foxn1 [Su(var) factor] and Ikaros for the transcriptional regulation of Notch-mediated T cell development (Bleul et al. 2006, Kleinmann et al. 2008, Ng et al. 2009), Pc-G members Bmi and Epc [Su(var) factor] as chromatin components in eliciting Hedgehog signals to repress the tumor suppressor *Ink4a* (*Arf*) locus (Bruggeman et al. 2005) or to discriminate brown/white adipose cell fate (Pospisilik et al. 2010), and class III Hdacs, or Sirtuins (for Sir2 orthologs), in attenuating TNF-α-modulated transcription of several NF-κB target promoters (Kawahara et al. 2009).

The above examples illustrate that a direct transcriptional readout of genes encoding mammalian Su(var) and E(var) factors may not be their main mode of function in the described signaling pathways. Rather, it appears that signaling pathways recruit Su(var) and E(var) proteins/enzymes to form alternate complexes and modulate their function and that of other effector molecules, primarily TFs and chromatin remodelers.

SU(VAR) AND E(VAR) FACTORS CAN RESPOND TO STRESS SIGNALS

Various stress signals (e.g., heat, heavy metals, UV-C, oxidative and hyperosmotic stress) induce transcription from pericentric Sat III (human) and probably also major satellite (mouse) repeats, and promote the formation of nuclear stress bodies (Valgardsdottir et al. 2008). Interestingly, the different stress signals are mediated via distinct TFs, e.g., heat shock by HSF1 and osmotic shock by TONEBP, and it remains to be determined whether these TFs have embedded binding sites in the pericentric DNA repeats of either human or mouse chromosomes. Intriguingly, SIRT1 can deacetylate HSF1, which allows for prolonged HSF1 binding to DNA (Westerheide et al. 2009). Also, Pias-dependent sumoylation of members of another class of chromatin-modifying enzymes, the poly-ADP-ribose polymerases

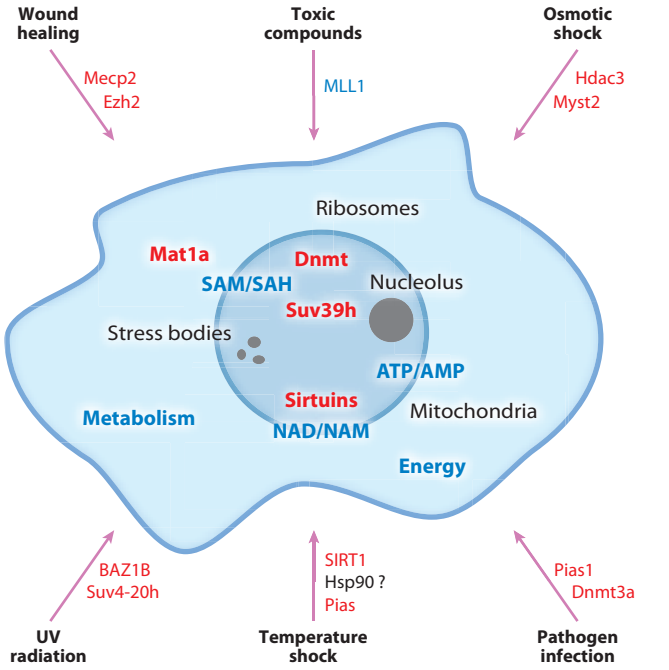

Figure 6

Stress signals to and metabolic modulation of chromatin. A diagram of a mammalian cell with the nucleus and other organelles is shown. Various external stress stimuli and metabolic fluctuations in the ratios of SAM/SAH, NAD/NAM, and ATP/AMP can adjust the activities of most of the indicated Su(var) (*red*) and E(var) (*blue*) factors.

(PARPs), upon heat shock has been described (Martin et al. 2009). In addition, in *Drosophila* the heat shock protein HSP90 directly interacts with and stabilizes the Trx-G member TRX at target promoters to maintain a transcriptionally active state (Tariq et al. 2009). Pharmacological abrogation (with radiciol, which blocks ATP association of HSP90) or genetic abrogation of the HSP90 chaperone results in TRX degradation and heritable phenotypic variation, and it is possible that similar stress mechanisms may also contribute to modulation of the mammalian TRX orthologs Mll/MLL (Ansari et al. 2009) (**Figure 6**).

Upon osmotic shock (e.g., sorbitol or high salt concentrations), Hdac3 variants are generated by both alternative splicing (Gray et al. 2003) and by caspase-7-mediated proteolytic cleavage of the C terminus (Xia et al. 2007). This truncated Hdac3 variant induces cell death by repressing the antiapoptotic c-*Jun* gene.

Osmotic shock also neutralizes the HAT activity of Hbo1/Myst2 by inducing stable interactions between upregulated p53 and Hbo1/Myst2 (Iizuka et al. 2008). This results in cell cycle arrest, as chromatin loading of the DNA replication licensing complex Mcm2-7 depends on acetylation by Hbo1/Myst2. Similarly, food contaminants such as mycotoxin have been shown to upregulate the *MLL1* gene (Ansari et al. 2009). Nickel ions, although general toxins, can inhibit jumonji-type KDMs via replacement of the Fe(II)-oxoglutarate cofactor, thereby altering H3K9me3 chromatin imprints at certain transgene locations (Chen et al. 2006).

DNA damage by double strand breaks (DSB) triggers a series of extensive chromatin reorganizations and repair signaling mechanisms (Callen et al. 2007, McKinnon & Caldecott 2007) to which BAZ1B (WSTF) was recently connected. In addition to its other roles in heterochromatin replication (Poot et al. 2004), BAZ1B also has an intrinsic tyrosine kinase activity that phosphorylates Y142 of H2A.X, which is the chief histone variant for DSB response (Xiao et al. 2009). Furthermore, deletion of the Suv4-20h enzymes, which convert H4K20me1 to H4K20me3 marks, results in increased sensitivity to DNA damage and delayed DSB repair, in part by weakening the chromatin association of the DNA repair factor 53BP1 (Schotta et al. 2008).

An unbiased reconstruction of the transcriptional networks that mediate pathogen response in primary mouse dendritic cells indicated that the Toll-like receptors (Tlrs) can distinguish between broad pathogen classes. This study also revealed approximately 25 core components in the control of pathogen response, including TFs, chromatin modifiers, and RNA binding modules (Amit et al. 2009). Intriguingly, Dnmt3a insulates against bacterial pathogens but weakens viral response by directly silencing the *interferon β1* gene. Similarly, PIAS-1 was shown to attenuate IFN-γ signaling in human macrophages (see **Figure 5**). This can explain why Pias1-null mice display hyperactive innate immune response (Liu et al. 2004).

Tissue damage/wound healing also induce a complex transcriptional readout that entails cell reprogramming. This involves downregulation of Pc-G function, as was shown for JNK-mediated repression, via AP1 TFs of the *Polycomb* gene during forced transdifferentiation of cell fates derived from *Drosophila* imaginal discs (Lee et al. 2005). In mice, skin injury reduces *Ezh2* and *Suz12* transcription in epidermis cells by simultaneously upregulating expression of *Jmjd3* and *Utx*, which encode H3K27me3 KDMs (Shaw & Martin 2009). This dual response annihilates repressive H3K27me3 marks and activates several target genes, among them *Myc* and *Egfr*, which are central to cell proliferation and facilitation of wound healing. Moreover, transdifferentiation of mouse myofibroblasts after wounding requires repression of cell type–specific genes by increased DNA methylation and recruitment of Mecp2 (Mann et al. 2007).

BALANCING SU(VAR) AND E(VAR) FUNCTION BY METABOLIC STATE

Approximately 50% of the identified Su(var) and E(var) factors are enzymes. All of these require cofactors [e.g., acetyl-Coenzyme A (CoA), nicotine amide dinucleotide (NAD), S-adenosyl methionine (SAM), ATP] and therefore are ideally suited to respond to external and intrinsic signals that monitor metabolic state (e.g., nutrient availability) and indicate energy consumption (e.g., caloric restriction) and cell proliferation conditions (e.g., enhanced rRNA transcription and ribosomal activity).

Fluctuations in ATP/ADP/AMP levels would primarily modulate the activity of Su(var) and E(var) factors that are components of chromatin remodelers (Atrx, Baz, Rsf, Lsh, reptin, Baf60, Brg1, Snf2h/Iswi) and DNA/RNA helicases (see **Supplemental Tables 1** and **2**, follow the **Supplemental Material link** from the Annual Reviews home

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page at **http://www.annualreviews.org**). Of note are further mammalian orthologs (e.g., Ppp1cc) of the SU(VAR)3–6 phosphatase, which can remove H3S10phos, thereby protecting the chromatin association of HP1 (Fischle et al. 2005). However, whereas oxidative phosphorylation (ox/phos) is prevalent in mitochondria, the broad abundance of ATP in the cell makes any specific ATP fluxes in the modulation of Su(var) and E(var) function difficult to examine.

Acetyl-CoA is the major cofactor for acetyltransferases (HATs) such as Hbo1/Myst2. Whereas class I and class II HDACs require only CoA as an acceptor molecule in deacetylation reactions, activity of the class III HDACs or Sirtuins (SIRT) is strictly dependent on NAD, as are the PARP enzymes (Yang & Sauve 2006). Whereas PARPs display no direct Su(var) or E(var) function, Sirtuins are prominent Su(var) enzymes and are among the most versatile transducers for epigenetic control to remove acetylation marks from histones and many nonhistone proteins in response to external signals and in sensing metabolic state (Vaquero & Reinberg 2009). NAD is recycled primarily by the NAD salvage pathway, in which the activity of several key metabolic enzymes is highly responsive to environmental signals, caloric restriction, and stress (Vaquero & Reinberg 2009, Yang & Sauve 2006). Interestingly, nicotinamide mononucleotide adenyltransferase-1 (Nmnat-1), which generates NAD, can directly be recruited to chromatin targets by Sirt1 (Zhang et al. 2009). This will increase the local NAD/nicotine amide mononucleotide (NAM) ratio, thereby precluding competitive inhibition of SIRT function by NAM. A particularly illuminating example is provided by the integration of circadian rhythms, in which oscillating NAD/NAM levels dictate Sirt1 over Clock (HAT) activity to regulate deacetylation-driven periodic degradation of Per2 and other TFs (Asher et al. 2008, Nakahata et al. 2008).

SIRT function also has indirect effects because its reactivity generates the metabolic intermediate O-acetyl-ADP-ribose (OAADPR), which can be bound by the Xi-enriched histone variant macroH2A1.1 to attenuate its function in chromatin regulation (Till & Ladurner 2009). Another indirect function and example for the complex interplay between chromatin-modifying enzymes is the SIRT1-dependent deacetylation of SUV39H1 at its SET domain, which enhances SUV39H1 H3K9me3 KMT activity and helps promote heterochromatin formation (Vaquero et al. 2007).

The rRNA loci in the nucleolus are also coregulated by SIRT activity, as the NoRC protein complex, which contains the H3K9me2 binder nucleomethylin, Suv39h1, and Sirt1, is involved in gene repression (McStay & Grummt 2008). In this context, Sirt1 strengthens the interaction of NoRC with promoter-associated RNA by removing acetylation from NoRC components (Zhou et al. 2009).

SAM is the major methyl donor for most methyltransferases (DNA, RNA, histone, and nonhistone) in the cell, and SAM metabolism is a classic paradigm for epigenetic regulation, as it may be dependent on metabolic state (Luka et al. 2009). *Su(z)5*, the gene encoding SAM synthetase, is a strong *Su(var)* modifier in *Drosophila* (Larsson et al. 1996). Activity levels of the human enzyme ortholog MAT1 are reduced in chronic liver diseases, and mice deficient for Mat1 develop hepatocellular carcinoma (Mato et al. 2008, Rountree et al. 2008). These molecular connections can explain dietary benefits that are associated with folate and vitamin B12 nutrient supplements (Miller et al. 2008). Higher availability of methyl donors (folate, choline, vitamin B12, and others) has been shown to increase DNA methylation and silence repeat-associated gene expression in mice (Waterland et al. 2006), which particularly affected epigenetic variation in the offspring of mothers who were fed a high folate diet (Dolinoy & Jirtle 2008, Dolinoy et al. 2006). Furthermore, increased methylation potential, i.e., higher SAM/S-adenosyl homocysteine (SAH) ratios in human serum, has been correlated with improved cognitive functions in Parkinson's disease (Obeid et al. 2009), whereas elevated SAH levels have been

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associated with increased cardiovascular risk (Castro et al. 2003).

Another fascinating mechanism to modulate SAM/SAH ratios would be the ability of certain RNA aptamers/riboswitches to capture SAM (J.X. Wang et al. 2008). This has thus far been shown only in bacteria, where SAM-binding riboswitches coregulate gene expression for SAM recycling. If they could also be identified in mammals, SAM-binding RNA molecules would provide an elegant epigenetic switch to adjust both the DNA and RNA (Goll et al. 2006) and the histone-methylating enzymes and to change local chromatin structure by connecting transcriptional output with the neutralization of a metabolite/cofactor.

Fluctuations in metabolic state would affect enzymatic activities in both the nucleus and cytoplasm. This is not restricted to histone and chromatin modifications, as was shown for Ezh2-mediated methylation in actin polymerization (Su et al. 2005) as well as for the early descriptions of cytochrome *c*– (Martzen et al. 1999) and rubisco-methylating enzymes (Klein & Houtz 1995) that were essential in the discovery of Suv39h KMTs (Rea et al. 2000).

ABERRANT SU(VAR) AND E(VAR) FUNCTION IN HUMAN DISEASE

With all these functions in chromatin biology and important roles during eukaryotic development and differentiation, it is not surprising that various forms of disease and cancer have been associated with nearly all human orthologs of the described *Su*(*var*) and *E*(*var*) genes (see **Supplemental Tables 3** and **4** for a full listing; follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**). Although these connections may in certain cases be only correlative, we have cross-referenced the available gene databases (NovoSeek, GeneCards, Genetic Association Database, and PubMed) to search for a more direct, gene-specific cause (i.e., point mutation, DNA deletion/translocation, or gene amplification) of a human disease. Of the approximately 70 human *SU*(*VAR*) and *E*(*VAR*) orthologs, at least 20 have DNA lesions that have been associated with a given disease. Therefore, they classify as genetic and heritable (familial) diseases. We have listed most of these known disorders in **Table 3**, with a preference for *SU*(*VAR*) genes that encode human orthologs of core heterochromatin components.

Heritable Diseases

Heritable diseases are indicated in bold in **Table 3**, with human disease-related references only, as provided by the databases. *ATRX* mutations are characterized by genital abnormalities, severe developmental delays, and mental retardation. ATRX is recruited to chromatin by MECP2, which is prevalent in neurons but not in other tissues (Nan et al. 2007). This connects the mental retardation phenotype of ATRX with Rett syndrome, which is caused by mutations in *MECP2*. MECP2 is a 5-me CpG binder, and Mecp2 deficiency can be corrected by providing intact Mecp2 function in a mouse model (Guy et al. 2007). Perturbed DNA methylation is also involved in the etiology of the ICF (immunodeficiency, centromere instability, and facial anomalies) syndrome that is caused by a recessive missense mutation in the *DNMT3B* (*Momme D14*) gene. Absence of DNMT3B function results in DNA hypomethylation of satellite 2 (Sat2) repeats, particularly in the pericentric heterochromatin of human chromosomes 1, 9 and 16. Williams-Beuren syndrome is caused by dominant mutations in the *BAZ1B* (*WSTF* and *Momme D10*) gene that manifest in mental retardation as well as dental and facial aberrations. BAZ1B interacts with SNF2H (ISWI and Momme D4) to form the WICH complex (Cavellan et al. 2006), which is targeted to replication foci by PCNA. Loss of BAZ1B function results in ectopic heterochromatin formation and globally reduced transcription (Poot et al. 2004). Mutations in the genes encoding the zinc-finger TFs SALL1 and SALL4 are the basis of dominant autosomal disorders that present with renal, ear, and limb malformations and are summarized

Table 3 Core *SU(VAR)* genes in human diseases

Gene	Disease	Cancer
SUV39H1 (*KMT1A*)	Facioscapulohumeral dystrophy (FSHD)	Retinoblastoma, lung adenocarcinoma, breast
HDAC1	Schizophrenia	Prostate, breast, kidney, gastric, colorectal, ovarian, endometrial
HDAC 2	Chronic obstructive pulmonary disease (COPD), asthma	
HMGA1	Diabetes	Breast, prostate, lung, pancreas, thyroid, ovarian, pituitary, gastric and lipomas
HMGA2	Silver-Russell syndrome	
TRIM28 (*TIF1B*)	–	Breast cancer
ATRX	**ATRX syndrome, α-thalassemia myelodysplasia syndrome**	Acute myeloid leukemia
***BAZ1B* (*WSTF*)**	**Williams-Beuren syndrome**	–
SALL1	**Townes-Brocks syndrome**	–
SALL4	**Okihiro syndrome**	Acute myeloid leukemia
MBD1	Autism	Prostate, lung, breast, intestine, colon, gastric
MECP2	**Rett syndrome**	–
DNMT3B	**ICF syndrome**	Colorectal, breast
DNMT1	Schizophrenia	Prostate, breast, lung, hepatocellular carcinoma, colorectal, gastric
	Late onset Alzheimer's disease (LOAD)	
EZH2 (*KMT6*)	–	Prostate, breast, bladder, liver, mantle-cell lymphoma, B cell non-Hodgkin's lymphoma
***EHMT1* (*GLP*)**	**9q subtelomeric deletion syndrome**	–

Listed are human diseases in which the core *SU(VAR)* gene has been associated. If a familial mutation in the *SU(VAR)* gene has been identified, this is shown in bold. Genetic linkages were validated using OMIM (PubMed). Disease-causative genetic lesions have also been identified for other *SU(VAR)* and *E(VAR)* genes including *NAP1L4*, *VIGILIN*, *FMR* and *MLL1*, *-2 (KMT2A, -2B)*, *JARID1C (KDM5C)*, and *LMNA*. For a full listing of *SU(VAR)* and *E(VAR)* genes in human diseases and cancer, refer to **Supplementary Tables 3** and **4** (follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**), which include links to the utilized databases and references.

as Townes-Brocks (*SALL1*) and Okihiro syndromes (*SALL4*). Interestingly, murine Sall1 and Sall4 are both required for the targeted activity of Whsc1/Nsd2 (a H3K36me3 KMT), suggesting an indirect connection of SALL1 and SALL4 to Wolf-Hirschhorn syndrome (Nimura et al. 2009). EHMT1 (GLP) is a euchromatic H3K9me2 KMT in which regional DNA mutations have been linked to 9q subtelomeric deletion syndrome.

Not indicated in **Table 2** but listed in **Supplemental Tables 3** and **4** (follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**) are other known disease-causative genes, such as *NAP1L1*, *VIGILIN*, *FMR1*, *JARID1C* (*KDM5C*), and *LMNA*. Furthermore, the prominent basis in mixed lineage leukemia and acute myeloid leukemia for the frequent translocations in the *MLL* genes that abrogate function of the catalytic SET domain has been well described (Ayton & Cleary 2001, Dou et al. 2005).

Candidate Diseases

These diseases are without familial inheritance. In facioscapulohumeral dystrophy (FSHD), contractions of the D4Z4 repeat region on chromosome 4q attenuate SUV39H1-mediated H3K9me3 and derepress putative candidate genes for muscular dystrophy (Zeng et al. 2009). However, the *SUV39H1* gene is located on the X chromosome. Hemizygous deletions including *HMGA1* were detected with DNA samples from diabetic patients and have been associated with decreased expression levels of the insulin receptor (Foti et al. 2005). Silver Russell Syndrome (SRS) is a growth retardation disorder with characteristic

Supplemental Material

facial malformations. SRS patients display microdeletions in chromosomal region 12q14, which includes the *HMGA2* gene (Spengler et al. 2010). Autism is a neurodevelopmental disease characterized by communication deficits and abnormal stereotyped behaviors. Due to the complexity of the disorder, no single causative gene has been discovered, although several single nucleotide polymorphisms (SNPs) in the *MBD1* gene have recently been identified in DNA preparations that were derived from cells of autistic patients (Cukier et al. 2010). Dysregulation of EZH2 has been associated with many types of human cancer and is probably a consequence of the highly dynamic upregulation of the *EZH2* gene following mitogenic/proliferative signals (Bachmann et al. 2006, Simon & Lange 2008).

Mouse models will be helpful to further define a more direct involvement of these candidate genes in disease. This is indicated for *Suv39h1*-null mice in accelerating tumor models of B cell lymphoma (Braig et al. 2005) as well as corroborated for *Hmga2* mutants (*pygmy*) in mouse dwarfism (Zhou et al. 1995); for *Mbd1−/−* mice displaying autistic-like phenotypes, possibly through an increase in serotonin receptor 2c levels (Allan et al. 2008); and for *Trim28* mutants that succumb to obesity (E. Whitelaw, personal communication) and to age-related anxiety (Jakobsson et al. 2008).

EPIGENETIC MODULATION OF COMPLEX HUMAN DISORDERS

Based on the delicate adaptabilities of SU(VAR) and E(VAR) function to changing environmental conditions, the distinction between purely heritable (genetic) diseases and more complex, non-Mendelian (epigenetic) disorders poses a difficult conundrum, and it is possible that certain *SU(VAR)* and *E(VAR)* genes could fall into both categories. Conspicuously, four gene classes (see **Supplemental Tables 3** and **4**; follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**) display a high incidence for more complex (in particular neurodegenerative and metabolic) disorders. These are the *HDAC*, *SIRT*, and *DNMT* gene families as well as genes encoding products that are involved in RNA processing, such as *FMR1* [all of these are *SU(VAR)* genes].

Examples of such epigenetic contributions of SU(VAR) enzymes to complex human disorders include DNMT1 in Alzheimer's disease (Mastroeni et al. 2008, S.C. Wang et al. 2008), DNMT1/DNMT3a (Zhubi et al. 2009) and HDAC1 (Sharma et al. 2008) in schizophrenia, SETDB1 (Ryu et al. 2006) and SIRT1 (Pallas et al. 2008) in Huntington's disease and diabetes (Liang et al. 2009), SIRT2 (Outeiro et al. 2007) in Parkinson's disease, and HDAC2 (Bhavsar et al. 2008) in asthma and allergic diseases. In all these cases, the disease-associated *SU(VAR)* genes displayed aberrant expression profiles without an identified DNA mutation in the gene locus. Even more convincingly, several of these disease symptoms could be ameliorated by small molecule inhibitors against DNMT, HDAC, and SIRT activity, demonstrating that epigenetic therapy has become a reality that extends well beyond cancer treatment (Batty et al. 2009, Gal-Yam et al. 2008, Jones & Baylin 2007, Zheng et al. 2008).

Although our deeper understanding of Su(var) and E(var) function could help to better diagnose and combat these complex human syndromes and may even allow for new therapeutic prospects for psychiatric disorders and depression (Holsboer 2008), there is also evidence for epigenetic contribution to habitual functions, such as long-term potentiation (LTP) of learning and memory. For example, in rat hippocampus, increased Dnmt1 levels facilitate fear conditioning and learning by silencing the *Pp1* memory suppressor gene, whereas pharmacological inhibition of Dnmt1 blocks LTP (Miller & Sweatt 2007). By contrast, attenuation of Hdac2 function by small molecule inhibitors promotes synaptic plasticity and facilitates memory output (Fischer et al. 2007, Guan et al. 2009).

The most direct epigenetic basis for human disease is perhaps provided by altered patterns of DNA methylation that have been associated

with genomic imprinting disorders (Feinberg et al. 2002, Y.H. Jiang et al. 2004) and found in aberrant silencing of tumor suppressor genes in cancer (Feinberg et al. 2006, Jones & Baylin 2007). But even these studies must be analyzed with great care to exclude SNPs because DNA methylation has been shown to be an epigenetic driver in promoting higher mutation rates as a consequence of spontaneous deamination of 5-me CpG (Eden et al. 2003, Shen et al. 1992).

One of the hallmarks of epigenetic control is to allow for highly variable adaptations of a genetically identical chromatin template. This is best exemplified by the temporal progression (during aging) of discordant DNA and histone methylation profiles between human monozygotic twins, particularly if they were exposed to varying lifestyles under different environmental conditions (Fraga et al. 2005). However, genome-wide association studies with DNA from somatic cells that were obtained from human twin cohorts indicate a persistent correlation between phenotypic variation and DNA sequence polymorphisms (Boomsma et al. 2002).

OUTLOOK

In this review we have detailed the molecular functions and biological roles of the currently known (composing ~15% of the predicted) mammalian Su(var) and E(var) factors that have been revealed by the PEV modifier screens in *Drosophila*. Although their analysis has uncovered basic principles and underlying mechanisms for how a dynamic chromatin landscape can facilitate lineage decisions, many more exciting insights and new pathway interactions can be anticipated from the full exploration of the entire complement of *Drosophila* and mouse/human *Su*(*var*) and *E*(*var*) gene products. Among those will be a deeper understanding of the functions of noncoding RNAs (Kapranov et al. 2007, Mercer et al. 2009), an even broader scale of chromatin modifications (Daujat et al. 2009, Kouzarides 2007), possible in vitro reconstitution (Francis et al. 2004, Trojer et al. 2007) and resolution of the in vivo physicochemical properties (Ghirlando & Felsenfeld 2008) of heterochromatin, and identification of novel classes of small molecule inhibitors that will, for example, be directed against KDMs and sumoylating (Fukuda et al. 2009) enzymes. Finally, the nearly endless adaptability of Su(var) and E(var) activities in response to physiological and pathological signals offers numerous pathways for epigenetic regulation and exposes them as central chromatin modulators as well as valuable therapeutic targets for the diagnosis of and pharmacological intervention in human disease.

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