Advances in chromatin remodeling and human disease
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Epigenetic factors alter phenotype without changing genotype. A primary molecular mechanism underlying epigenetics is the alteration of chromatin structure by covalent DNA modifications, covalent histone modifications, and nucleosome reorganization. Remodeling of chromatin structure regulates DNA methylation, replication, recombination, and repair as well as gene expression. As these functions would predict, dysfunction of the proteins that remodel chromatin causes an array of multi-system disorders and neoplasias. Insights from these diseases suggest that during embryonic and fetal life, environmental distortions of chromatin remodeling encode a 'molecular memory' that predispose the individual to diseases in adulthood.

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Abbreviations
A acetyl group
ATRX α-thalassemia X-linked mental retardation
BAZ1B bromodomain adjacent to zinc finger domain, 1B
BDNF brain-derived neurotrophic factor
BRG1 brahma-related gene 1
BRM brahma
CSB Cockayne syndrome, type B
HDAC histone deacetylase
ISWI imitation switch
MECP2 methyl-CpG-binding protein 2
mSin3A mammalian Sin3 homolog A
OMIM Online Mendelian Inheritance in Man
RNAP II RNA polymerase II
RXR retinoid X receptor
VDR vitamin D receptor
VDRE vitamin D responsive element
WICH BAZ1B/WSTF-ISWI chromatin remodeling complex
WINAC BAZ1B/WSTF including nucleosome assembly complex
WSTF Williams syndrome transcription factor

Introduction
Within the context of human disease, phenotypic variation has typically been attributed to differences in genetic background and the influences of environment on that genetic background. In isogenic populations of model organisms, however, phenotypic variations persist [1,2**]. These studies of isogenic organisms showed that changes in chromatin structure and DNA methylation, arising stochastically or in response to physiologic stress, account for this variation and can be transmitted through mitosis and meiosis [1,2**,3]. Medically, these observations suggest a role for epigenetics in complex diseases and a mechanism by which exposure of a gamete or embryo to stresses can predispose that individual to adult disease [4].

The human genome is assembled into chromatin, which consists of DNA and protein condensed into nucleoprotein complexes [5]. The fundamental packaging unit of chromatin is the nucleosome, 147 base pairs of DNA wound twice around an octamer core of histones (two each of the H2A, H2B, H3 and H4 histones). The position and density of histone octamers along the DNA are mediated by ATP-dependent chromatin remodeling complexes that bind DNA and use the energy from ATP hydrolysis to move the histone octamers among and along DNA molecules [6,7]. The affinity of histones for DNA and chromatin-associated proteins is controlled by acetylation, methylation, phosphorylation, poly-ADP ribosylation, and ubiquitination of histone amino termini [8]. These modifications and the positioning of histones organize the genome into either open or condensed chromatin and thus regulate the accessibility of DNA for transcription, methylation, recombination, replication, and repair. The positioning and modification of the histones form a histone code or epigenetic 'memory' that is passed from mother cell to daughter cells [9].

Mutations affecting the function and targeting of chromatin-remodeling complexes generally cause cancers or multi-system developmental disorders. The multi-system nature of these single-gene disorders can be explained by deregulation of chromatin structure at many loci. In this review, we focus on the recent advances in our understanding of cancers and Mendelian disorders ascribed to defects of ATP-dependent chromatin remodeling and histone acetylation and deacetylation.

ATPase-dependent chromatin remodeling and disease
ATP-dependent chromatin remodeling complexes contain several subunits, one of which is a SWI/SNF-related ATPase (Table 1). The amino and carboxyl domains flanking the ATPase domain of the SWI/SNF-related proteins allow association with other proteins in the
The SWI/SNF-related ATPase derives specificity for chromatin domains through these interactions. Structural modification of chromatin is essential for all processes requiring access to nuclear DNA (Figures 1–3).

**SMARCB1, BRG1, and BRM: cancer**

Mutations of **SMARCB1** (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily B, member 1) and **BRG1** (brahma-related gene 1), and reduced expression of **BRM** (brahma) have been identified in primary tumors and tumor-derived cell lines [8]. The associated neoplasms for **SMARCB1** mutations include rhabdoid tumors and chronic myeloid leukemia; for **BRG1** mutations, they include lung, breast, and prostate cancer; and for **BRM** repression, they include lung, breast, and prostate cancer. Characteristic of tumor suppressors, the **SMARCB1** and **BRG1** mutations are biallelic and the reintroduction of the respective wild-type protein into tumor cell lines induces senescence. Also, reintroduction of **BRG1** alters expression of genes involved in cell-cycle regulation, cell adhesion, and cell migration [10,11*,12]. **SMARCB1**, **BRG1**, and **BRM** regulate cell growth and gene expression through activation of the retinoblastoma protein and, additionally for **BRG1** and **SMARCB1**, through interaction with **BRCA1** [10,13]. These studies suggest that **SMARCB1**, **BRG1**, and **BRM** inhibit neoplasia by transcriptional regulation of genes involved in cellular proliferation.

**BRG1 and BRM: Williams syndrome**

In addition to tumorigenesis, the **BRG1**- and **BRM**-associated chromatin remodeling complexes have been implicated indirectly in the pathology of Williams syndrome (OMIM 194050) [14**]. Williams syndrome, which is caused by heterozygosity for a microdeletion at 7q11.2, is an autosomal dominant disorder characterized by supravalvular aortic stenosis, peripheral pulmonary arterial stenoses, dysmorphism, mental and growth deficiency, aberrant vitamin D metabolism and hypercalcemia [15]. The transcription factor encoded by **BAZ1B** (bromodomain adjacent to zinc finger domain, 1B), a gene deleted in Williams syndrome, recruits **BRG1** and **BRM** with their associated chromatin-remodeling factors to vitamin D regulated promoters (Figure 1i) [14**,16,17]. When reintroduced into cell lines from Williams syndrome patients, **BAZ1B** restores vitamin D-responsive transcription [14**]. **BAZ1B** also interacts with **ISWI**, a SWI/SNF-related ATPase, to form a chromatin-remodeling complex that participates in DNA replication [18] (Figure 3). On the basis of these findings, aberrant chromatin remodeling might play a key role in the pathophysiology of Williams syndrome.

**ATRX: ATRX syndrome and α-thalassemia myelodysplasia syndrome**

Mutations in **ATRX** (α-thalassemia X-linked mental retardation; OMIM 301040) cause several X-linked mental retardation syndromes that also feature facial dysmorphism, urogenital defects, and α-thalassemia [19]. In males, somatic mutations of this gene also cause α-thalassemia myelodysplasia syndrome (OMIM 300448) [20*,21]. The **ATRX** chromatin-remodeling complex contains the Daxx transcription cofactor [22**]; thus, Daxx may target **ATRX**-mediated chromatin remodeling to specific promoters (Figure 1ii). Other studies demonstrated that the **ATRX** protein resides predominantly in repetitive DNA and that mutation of **ATRX** causes aberrant methylation of repetitive DNA elements [23,24]. Together these data suggest that **ATRX** is involved in transcription and the regulation of repressed chromatin.

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**Table 1**

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<thead>
<tr>
<th>Mammalian ATPase-dependent chromatin-remodeling complexes.</th>
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<tbody>
<tr>
<td>SNF2-like subfamily</td>
<td>Complex</td>
</tr>
<tr>
<td><strong>BRG1</strong>, <strong>BRM</strong>, <strong>SMARCB1</strong></td>
<td><strong>BRG1</strong>, <strong>BRM</strong>, bBAF, hRSC/PBAF, WINAC</td>
</tr>
<tr>
<td><strong>ISWI</strong>-like subfamily</td>
<td><strong>hISWI</strong> (hSnf2h/hSnlf2)</td>
</tr>
<tr>
<td>CHD subfamily</td>
<td><strong>CHD3</strong>, <strong>CHD4</strong></td>
</tr>
<tr>
<td>Other SNF2-related proteins</td>
<td><strong>ATRX</strong></td>
</tr>
<tr>
<td><strong>ERCC6</strong></td>
<td><strong>CSB</strong></td>
</tr>
<tr>
<td><strong>SMARCAL1</strong></td>
<td>Unknown</td>
</tr>
</tbody>
</table>

ACF, ATP-utilizing chromatin assembly and remodeling factor; ATRX, α-thalassemia X-linked mental retardation; BAF, Br-1 associated factor; BAZ1A, bromodomain adjacent to zinc finger domain, 1A; BRG1, brahma-related gene 1; BRM, brahma; CHD, chromo-helicase-DNA-binding protein; CHRAC, chromatin accessibility complex; CSB, Cockayne Syndrome, type B; ERCC6, excision repair cross-complementing rodent repair deficiency, complementation group 6; ISWI/ISW, imitation switch; NoRC, nucleolar remodeling complex; NURD, nucleosome remodeling and histone deacetylation; RSF, remodeling and spacing factor; SMARCAL1, SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A-like 1; SMARCB1, SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily B, member 1; SWI/SNF, switching/sucrose non-fermenting; WICH, WSTF-ISWI chromatin remodeling complex; WINAC, WSTF including nucleosome assembly complex; WSTF, Williams syndrome transcription factor.
ERCC6: cerebro-oculo-facio-skeletal syndrome and Cockayne syndrome, type B

Mutations in ERCC6 (excision repair cross-complementing rodent repair deficiency, complementation group 6) cause cerebro-oculo-facio-skeletal (COFS: OMIM 214150) syndrome and Cockayne syndrome, type B (CSB: OMIM 133540). These diseases are characterized by neurological degeneration, growth failure, cataracts, and UV-sensitivity. Based on the yeast homologue RAD26, ERCC6 promotes transcriptional elongation by RNA polymerases I, II, and possibly III and also enhances transcription-coupled DNA repair and some forms of global genome base excision repair (Figure 2) [25]. RAD26 promotes transcriptional elongation by modifying chromatin structure to minimize secondary DNA structures [26], and in the event of impassable DNA damage, it promotes...
removal of the RNA polymerase complex to allow the repair enzymes access to the DNA [27,28]. The mechanism by which RAD26 enhances global genome base excision repair of oxidative damage is undefined. These multiple ubiquitous functions of this ERCC6 homolog provide a nascent explanation for the severe multi-system nature of COFS and CSB.

**SMARCAL1: Schimke immuno-osseous dysplasia**

Mutations in **SMARCAL1** (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A-like 1) cause Schimke immuno-osseous dysplasia (SIOD: OMIM 242900), an autosomal recessive disorder of T-cell immunity, spondyloepiphyseal dysplasia, renal failure, hypothyroidism, episodic cerebral ischemia, and bone marrow failure. Consistent with its putative role in chromatin remodeling, the SMARCAL1 protein binds nucleosomes but not isolated histone proteins [29]. Although the function of SMARCAL1 has not been defined biochemically, clinical findings suggest that SMARCAL1 may regulate a subset of genes involved in the proliferation of affected tissues [30].

In summary, mutations within the genes encoding SWI/SNF-related enzymes as well as other components of ATP-dependent chromatin remodeling complexes cause a wide range of disease phenotypes. These phenotypes illustrate the role of chromatin remodeling in the...
regulation of gene expression, DNA repair, and DNA replication (Figures 1–3).

**Histone acetylation and disease**

Generally, SWI/SNF-related ATPases and histone acetyltransferases (HATs) and deacetylases (HDACs) cooperate in chromatin remodeling; at some promoters, a SWI/SNF-related ATPase recruits a HAT or a HDAC, whereas at other promoters, a HAT or a HDAC recruits a SWI/SNF-related ATPase. Like SWI/SNF-related ATPases, HATs and HDACs are members of multiprotein complexes; the interacting proteins such as methyl-CpG-binding proteins (MECPs) target these complexes to genomic loci (Figure 1iii).

Histone acetylation reduces the avidity of DNA–histone interactions by increasing the negative charge on histone amino-termini and thereby the repulsion between nucleosomes and between DNA and histones. The resulting relaxed chromatin conformation promotes access of other DNA-binding proteins. Acetylation of large chromatin domains leads to partial decondensation of chromatin and defines a region of transcriptional competence, but transcriptional activation of a promoter within a permissive region requires additional acetylation targeted to the promoter histones. Histone acetylation and deacetylation is modulated by the interplay between HATs and HDACs (Table 2), and imbalances of this process cause multi-system diseases and cancers.

**CREBBP: Rubinstein Taybi syndrome and polyglutamine neurodegeneration**

Mutations in the CREBBP (cAMP-responsive element binding protein [CREB] binding protein) gene cause Rubinstein Taybi syndrome (RTS; OMIM 180849), an autosomal dominant disorder of growth retardation, facial abnormalities, broad thumbs and toes, and mental retardation. As a HAT, CREBBP promotes the decondensation of chromatin and facilitates transcription through recruitment of several transcriptional regulators including c-Myc, c-Fos, c-Jun, and CREB [31,32]. The mechanisms by which decreased CREBBP dosage causes malformations remain obscure, although the mental retardation in RTS may be explained partially by the role of CREBBP-mediated histone acetylation in synapse plasticity and long-term memory [33].

In polyglutamine neurodegenerative disorders such as Huntington disease, the polyglutamine repeat expansion forms neuronal nuclear inclusions. CREBBP is recruited to nuclear inclusions formed by pathologic repeats of polyglutamine in cell culture, transgenic mice, and human postmortem tissue [34,35]. As a result, little endogenous CREBBP is found outside the inclusion, suggesting nearly complete sequestration [35]. Affirming the association between CREBBP sequestration and the neurodegeneration associated with these diseases, overexpression of CREBBP inhibits polyglutamine-induced neurodegeneration in cell culture and *Drosophila* models of polyglutamine repeat diseases [35,36]. These data suggest that by modification of chromatin structure, CREBBP regulates the expression of gene products necessary for neuronal survival and synapse function.

**CREBBP and EP300: cancer**

Similar to ATPase-dependent chromatin-remodeling complexes, some HATs are deregulated in human neoplasias and play crucial roles in oncogenesis [37,38]. CREBBP and e1a-binding protein p300 (EP300) suppress tumor formation. Mutations of *EP300* have been identified in human primary tumors as well as in tumor cell lines; in mice, mutations of *CREBBP* predispose to hematologic tumors [39]. In addition, errant targeting and recruitment of CREBBP and EP300 have been associated with aggressive human acute myeloid leukemia [40]. Errant targeting of CREBBP and EP300 occurs through chromosomal translocations producing fusion proteins of CREBBP or EP300 with zinc finger protein 220 (ZNF220), whereas aberrant recruitment of CREBBP occurs when a chromosomal translocation causes expression of a fusion protein consisting of nuclear receptor co-activator 2 and ZNF220.

**Table 2**

<table>
<thead>
<tr>
<th>Mammalian HATs and HDACs.</th>
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<tbody>
<tr>
<td>HAT</td>
<td>HDAC</td>
</tr>
<tr>
<td>GNAT family</td>
<td></td>
</tr>
<tr>
<td>PCAF, GCN5L2</td>
<td>Class I-3, HDAC8</td>
</tr>
<tr>
<td>CREBBP/EP300 family</td>
<td></td>
</tr>
<tr>
<td>CREBBP, EP300</td>
<td>Class II</td>
</tr>
<tr>
<td>MYST family</td>
<td></td>
</tr>
<tr>
<td>HTATIP, ZNF220, HBO1, MORF, MYST1</td>
<td></td>
</tr>
<tr>
<td>TAFII 250 family</td>
<td>TAFII 250</td>
</tr>
<tr>
<td>SRC family</td>
<td></td>
</tr>
<tr>
<td>ACTR, SRC1, SRC3, NCOA2</td>
<td></td>
</tr>
<tr>
<td>Other HATs</td>
<td></td>
</tr>
<tr>
<td>TCF2</td>
<td></td>
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<tr>
<td>GTF3C1</td>
<td></td>
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<tr>
<td>ACTR, activin receptor; CREBBP, CREB-binding protein; EP300, e1a-binding protein p300; GCN5L2, general control of amino-acid synthesis 5-like 2; GNAT, GCN5-related acetyltransferase; GTF3C1, general transcription factor 3c, polypeptide 1; HAT, histone acetyltransferase; HBO, histone acetyltransferase binding to ORC; HDAC, histone deacetylase; HTATIP, HIV Tat interactive protein; HIV tat, human immunodeficiency virus type I trans-acting transcription factor; MORF, MOZ-related factor; MOZ, monocytic leukemia zinc finger protein; MYST, MOZ, YBF2/SAS3, SAS2, TIP60 protein family; NCOA2, nuclear receptor coactivator 2; ORC, original recognition complex; PCAF, EP300/CREBBP-associated factor; SIRT1, sirtuin; SRC, steroid receptor coactivators; TAF, TATA box-associated factors; TCF2, transcription factor 2; ZNF220, Zinc finger protein 220.</td>
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</table>
MECP2: Rett syndrome
Mutations of MECP2 cause Rett syndrome (OMIM 312750), neonatal-onset encephalopathy, mental retardation, autism, and an Angelman-like syndrome [41]. MECP2 binds methylated promoter sites and recruits the mSinA/HDAC1,2 co-repressor complex [42] and thereby mediates dynamic repression of gene expression [43,**,44,**,45**] (Figure 1iii). These data suggest that the pathology associated with MECP2 mutations results from inappropriate gene expression, and consistent with this hypothesis, histone H4 is hyperacetylated in patients with MECP2 mutations [46]. Dysregulation of brain-derived neurotrophic factor (BDNF), a MECP2 target gene, could account for some of the neuropathology because BDNF plays crucial roles in neuronal survival, development, and plasticity [43,**,44**].

HDAC activation: cancer
Abnormalities of global and targeted histone acetylation have been associated with several human neoplasms. In acute promyelocytic leukemia, acute lymphocytic leukemia, and non-Hodgkins lymphoma, mutations errantly activate and target interacting HDACs [8]. In human gastrointestinal cancers, overall histone acetylation is reduced [47]. Such inappropriate HDAC activity is predicted to cause transcriptional repression and lead to neoplastic transformation. Consistent with this hypothesis, inhibitors of HDAC function induce many tumors to stop proliferating and express differentiated characteristics; molecularly, these phenotypic changes are reflected by alterations in the expression profile of genes that are involved in cell-cycle control, apoptosis, DNA repair, and proteosome function [48,49*].

In summary, these associations between aberrant histone acetylation and deacetylation and various diseases suggest the importance of these processes for neuronal plasticity and control of cellular proliferation. In general, the associated developmental disorders generally result from inactivation of HAT or HDAC activity, whereas associated neoplasms result from activation, misexpression, or aberrant targeting.

Conclusions
The studies of chromatin structure in model organisms are the foundation for our investigation of the role of chromatin structure and epigenetics in human biology and disease. Such studies have illustrated the critical roles of chromatin remodeling in sequential gene activation and inactivation during development, differentiation, apoptosis, and homeostasis. Consistent with these observations in non-primates, the multi-system disorders and neoplasias discussed above implicate chromatin remodeling in nearly all aspects of human growth and development.

The phenotypic variations associated with mutations in chromatin-remodeling factors reflect the functional diversity, redundancy, and environmental modulation of the affected processes. Chromatin-remodeling factors are frequently components of several different complexes. As a consequence, the spectrum of mutations that affect protein–protein interactions could give rise to multiple phenotypes because each mutation could differentially affect each complex. Additionally, slight variations in the function of redundant and cooperative pathways could also contribute to the phenotypic diversity. For example, because the regulation of gene expression is a cooperative effect of ATP-dependent chromatin remodeling, covalent histone modifications, and DNA methylation, mutations in one process could be modified by slight variations in the activity of the complementary processes.

The multi-system character and variable expressivity of the Mendelian disorders discussed above suggest that perturbations of chromatin remodeling play a significant role in complex human diseases such as syndrome X (type 2 diabetes, cardiovascular disease, and hypertension). By epidemiologic studies, syndrome X is linked with poor prenatal nutrition and low birth weight [50]. Explanation of this phenomenon requires the compromised nutrition of the embryo or fetus to alter programming for organ development and physiologic homeostasis without changing the genotype. In *Drosophila*, physiologic stress alters chromatin structure and these epigenetic changes can be transmitted between generations and subjected to selective pressure [2**]. Thus chromatin remodeling, whether by DNA methylation or histone modification, provides a mechanism for prenatal stress predisposing to adult human diseases by altering gene expression in a heritable manner [51]. The continued pursuit of *in vitro* and *in vivo* studies of chromatin remodeling and the influences of environment on this remodeling will address this possibility.

In summary, the importance of chromatin remodeling in human biology, Mendelian disease, and somatic tumors has become increasingly apparent during the past few years. The current studies of model organisms excitingly suggest that heritable epigenetic variation will account for a portion of the phenotype in complex diseases and raise questions concerning the contribution of epigenetic variation to quantitative traits in general. In particular, do the increasing incidences of diseases such as asthma, syndrome X, and some neoplasias arise in part from environmental influences and the selection for epigenetic traits? Answering these questions is essential for defining precisely the molecular pathophysiology and scope of chromatin remodeling and epigenetics in human disease and will come through characterizing the fundamental epigenetic processes of chromatin remodeling in model organisms and directed human studies.

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manuscript. We apologize to those whose work could not be cited directly due to reference limitations and the scope of the review.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


The authors report phenotypic variation in an isogenic Drosophila strain. Their data suggest that reduced activity of the heat shock protein 90 induces a heritable alteration in the chromatin. This study provides further evidence for regulation of phenotype by epigenetic responses to the environment.


This paper shows that cell growth can be arrested upon the reintroduction of BRG1 into a breast tumor cell line that contains a nonsense mutation in exon 10 of BRG1. Comparison of the mRNA expression profiles in the presence and absence of reintroduced BRG1 identified several novel BRG1-regulated genes.


When compared to germline mutations of ATRX, somatic mutations in ATRX cause an unexpectedly severe hematological phenotype in males: myelodysplasia associated with α-thalassemia (ATMDS).


This paper presents the hypothesis that CBP acts as an oncogenic factor, rather than as a tumor suppressor in myeloid transformation by ZNF220–TIF2. The authors made a series of deletion mutants of ZNF220–TIF2 and measured their transforming activities and concluded that recruitment of active CBP is required for ZNF220–TIF2-induced transformation.
