NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH Public Access
Author Manuscript
Biochim Biophys Acta. Author manuscript; available in PMC 2011 January 1.

Published in final edited form as:
Biochim Biophys Acta. 2010 ; 1799(1-2): 3. doi:10.1016/j.bbagrm.2009.09.001.

HMG Nuclear Proteins: Linking Chromatin Structure to Cellular Phenotype

Raymond Reeves
Washington State University, School of Molecular Biosciences, Biotechnology/Life Sciences Bldg., Rm. 143, Pullman, WA 99164-7520

I. Summary

Although the three families of mammalian HMG proteins (HMGA, HMGB and HMGN) participate in many of the same nuclear processes, each family plays its own unique role in modulating chromatin structure and regulating genomic function. This review focuses on the similarities and differences in the mechanisms by which the different HMG families impact chromatin structure and influence cellular phenotype. The biological implications of having three architectural transcription factor families with complementary, but partially overlapping, nuclear functions are discussed.

II. Introduction

The 'High Mobility Group' (HMG) non-histone proteins occupy a unique niche in vertebrate biology. The distinctive constellation of ways they coordinate and facilitate DNA-directed nuclear processes, and thereby influence cellular phenotypes, sets them apart from all other regulatory molecules. Members of the three families of HMG proteins (HMGA, HMGB and HMGN; [22]) are accessory 'architectural factors' involved in modulating nucleosome and chromatin structure and orchestrating the efficient participation of other proteins in such vital nuclear activities as transcription, replication and DNA repair. In normal cells expression of HMG proteins is highly regulated and influenced by both developmental and environmental factors. Absence, mutation or aberrant levels of expression of HMG proteins can alter the phenotype of cells and lead to developmental abnormalities and disease. Nevertheless, individual HMG proteins are not essential for cell viability owing to the partially over-lapping and compensatory functions of the proteins within a given HMG family. Comprehensive reviews on all three HMG families have recently appeared and readers are referred to these, and other articles in this volume, for in-depth coverage of the known biological functions of these proteins [6,14,15,17,[23],[25],33,71,132–135].

The coverage here is more restricted and focuses primarily on similarities and differences in the ways the various HMG protein families coordinate changes in chromatin structure with alterations in gene expression and the phenotypic state of cells under normal and abnormal conditions. I apologize in advance to the many researchers whose excellent work in this area may not have been covered.

Tel: (509) 335-1948; Fax: (509) 335-4159; reevesr@wsu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

III. HMG Proteins: Mobile Modulators of Chromatin Structure and Cellular Phenotype

The term "High Mobility Group" was originally coined for the HMG proteins because of their unusual solubility properties, their small size and their rapid mobility, relative to other chromatin proteins, during gel electrophoretic separations [1]. Fortuitously, recent real-time imaging experiments with living cells have demonstrated that, as their name implies, HMG proteins are indeed highly mobile within the nucleus where they are part of a dynamic network of architectural components that modulate the structure of chromatin and, thereby, affect multiple DNA-dependent activities [30,31,61,62,119]. All HMG proteins have similar biochemical and biophysical properties and each is characterized by the presence of a long, negatively charged carboxy terminal tail that serves a regulatory function(s) [27,61]. The HMG families also participate in many common biological processes such as involvement in embryonic development, regulation of transcription and modulation of DNA repair. Nevertheless, the HMGA, HMGB and HMGN families are distinguished from each other by their unique DNA-binding motifs, by their preferred binding substrates, by the changes they induce in their substrates and by the different subsets of cellular processes they influence. The distinctive constellation of features that characterize different HMG families determines how individual proteins differentially affect chromatin structure, nuclear metabolism and cellular phenotypes.

IV. HMGA Proteins and Localized Nucleosome Structure

The canonical DNA-binding domain of the HMGA family (formerly called HMG-I/Y) is a palindromic amino motif called the 'AT-hook' that binds preferentially to the minor groove of short stretches of A/T-rich B-form DNA via recognition of structure rather than nucleotide sequence [138]. In mammals there are two sub-families of HMGA proteins, HMGA1 and HMGA2 [132]. The HMGA1 sub-family consists of three proteins (HMGA1a, HMGA1b and HMGA1c) that are produced by translation of alternatively spliced transcripts derived from a single gene. The HMGA2 sub-family, on the other hand, contains only one protein that is coded for by a different gene. All HMGA proteins contain three AT-hooks except for the rare HMGA1c variant which only has two. A unique feature of the HMGA proteins is that, as free molecules, they are disordered random coils and only assume any type of defined secondary structure after binding to DNA or other substrates [73]. This intrinsic flexibility, combined with a disordered-to- ordered structural transition following substrate binding, is thought to play an important role in allowing the HMGA proteins to participate in a wide variety of biological processes [45,133]. One of the best characterized biological roles of the HMGA proteins is in coordinating the formation of multi-subunit, stereospecific protein-DNA complexes called enhanceosomes [99] on A/T-rich promoter regions of certain inducible genes during their transcriptional activation [48,157]. HMGA proteins can bend, straighten, unwind and supercoil DNA substrates *in vitro* without requiring energy (i.e., ATP) input, capabilities that likely contribute to their ability to participate in enhanceosome formation and the regulation of gene expression *in vivo* [135].

In addition to recognizing the structure of the narrow minor groove of A/T-rich DNA as a target for binding, HMGA proteins also recognize and bind to non-B-form DNAs with unusual structural features. These binding substrates include: synthetic four-way and three-way junctions [66,68], bent and supercoiled DNAs [110], base-unpaired regions of A/T-rich DNA [91] and distorted or flexible regions of DNA on isolated nucleosome core particles [126, 139,141]. In the case of nucleosomes, HMGA binding induces localized changes in the rotational setting of DNA on the surface of reconstituted core particles, a restricted form of chromatin remodeling that is not driven by ATP hydrolysis [141]. Peptide domain swap

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

experiments employing hybrid recombinant proteins have demonstrated that the AT-hooks are the regions of HMGA proteins responsible for nucleosome binding [12].

The AT-hook motif is highly conserved in evolution from bacteria to humans and is found in one or more copies in a large number of other, non-HMGA, proteins, many of which are transcription factors or are involved in chromatin remodeling [8]. For example, AT-hook peptide motifs are essential components of the multi-protein, ATP-dependent chromatin remodeling complexes or ‘machines’ found in yeast, slime mold, insect, plant and mammalian cells. Specifically, AT- hooks are found in: the *Rsc-1* and *Rsc-2* proteins of the *Saccharomyces* RSC chromatin remodeling complex [28]; the multifunctional CbfA remodeling protein of *Dictyostelium* [94]; the largest protein subunit of the NURF chromatin remodeling complex of *Drosophila* [170]; the SPLAYED protein of *Arabidopsis* [155]; the APRIN chromatin remodeling protein of mammals [96]; the human MRN remodeling complex involved in double strand break repair [167]; and both the BRM/SNF2α and the BRG-1/SNF2β ATPase proteins of the human SWI/SNF-like complexes [21,150]. Furthermore, and most importantly, site-specific mutagenesis studies have demonstrated that when the AT-hook peptide motifs of the human BRG1 protein are deleted or mutated, both the nucleosome-binding and the ATP-dependent chromatin remodeling activities of the SWI/SNF complex are destroyed or greatly attenuated [21]. Together these findings suggest that most, if not all, of the major ATP-dependent chromatin remodeling complexes in eukaryotic cells contain proteins with functional AT-hooks that are involved in binding to nucleosomes in a manner similar to that of the HMGA proteins themselves. It thus seems likely that *in vivo* there is an intimate, and perhaps reciprocal, interaction between the AT-hook motifs of the HMGA proteins and those of the ATP-dependent chromatin remodeling machines in nuclear processes that require alterations in nucleosome structure.

There are numerous cases in which HMGA proteins have been linked to localized changes in chromatin structure that are coupled with concomitant alterations in the phenotypic characteristics of cells. Perhaps the best documented example involves the complex molecular and phenotypic cellular changes that follow virus infection of cells. Healthy cells do not normally produce type I interferons (IFN-α and IFN-β), secreted proteins of the innate immune response that interfere with viral replication and help block the spread of viruses to uninfected cells. Soon after viral infection, however, cells undergo a marked phenotypic change and start producing copious quantities of IFN-β followed about 24 hours later by a rapid postproduction turnoff. Double-stranded RNA produced during the viral life cycle induces assembly of an HMGA-containing enhanceosome on the promoter of the IFN-β gene and the initiation of mRNA transcription [157].

The intricate mechanics and multiple steps involved in the assembly/disassembly of the IFN-β enhanceosome during interferon turn on/off have been described in detail [5,99]. HMGA1 is a key player in coordinating enhanceosome assembly/disassembly and initiates the transcription activation process by binding to, and straightening, a short run of A/T-residues in naked DNA located between two well-positioned nucleosomes on the IFN-β promoter [5, 47,157,172,173]. The bound HMGA1 then assists in recruiting a number of transcription factors (IRF-1, p50, p65 and ATF-2/c-Jun) to an array of binding site on the naked DNA and coordinates the formation of the enhanceosome [172]. The stereospecific surface of the enhanceosome then recruits the GCN5 histone acetyltransferase (HAT) complex which acetylates histones on both of the adjacent nucleosmes but without altering their positions. GCN5 also acetylates a specific residue (K71) in the HMGA1 protein which strengthens the protein-protein interactions within the enhanceosome and is crucial for maintaining its stability during the transcription process [99,100]. Next, a SWI/SNF nucleosome remodeling complex and another HAT enzyme (CBP) are recruited to the enhanceosome. As a result of SWI/SNF remodeling activity, one of the two positioned nucleosomes moves or ‘slides’ 37 bp

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

downstream on the promoter, unmasking the TATA box and allowing TBP/RNA polymerase II holoenzyme recruitment and initiation of robust mRNA transcription [92,93]. Continuous acetylation of K71 on HMGA1 is required for transcription since deacetylation of this residue followed by acetylation of an adjacent lysine (K65) on the proteins by CBP leads to enhanceosome disruption and the termination of transcription [102–104]. Thus, dynamic acetylation/deacetylation of specific residues on the HMGA1 protein has been proposed to act as a regulatory 'switch' controlling the stabilization/disruption of the IFN-β enhanceosome. A potential caveat to this idea comes, however, from recent structural studies which have provided a complete atomic model of the protein-DNA interface of the IFN-β enhanceosome (reviewed in [115]). These studies demonstrate that the fully assembled enhanceosome cannot accommodate the HMGA1 protein since the minor grooves of all of its potential binding sites are either sterically blocked by other proteins or are too narrow to accommodate binding of the protein's AT-hooks. Therefore HMGA1 is unlikely to be a part of the final enhanceosome assembly. A possible explanation for this apparent discrepancy is that HMGA1 acts as a DNA molecular chaperone during enhanceosome assembly and, following its formation, dissociates from the final complex. Such a 'hit-and-run' mode of transcriptional regulation has been proposed for the HMGB family of proteins [6], as will be discussed below.

HMGA proteins have also been linked to localized changes in chromatin/nucleosome structure and alterations of cellular phenotype during induction of gene transcription in activated T lymphocytes. An adaptive immune response is initiated when naïve resting T lymphocytes encounter their corresponding antigen on the surface of antigen-presenting cells that also express co-stimulatory molecules. As a result of such interactions, T cells become activated and undergo marked phenotypic changes termed blast formation, a process that is critically regulated by the coordinate expression of cytokine genes. A key event in the initial activation of T cells is the production of the cytokine IL-2 and it's high affinity receptor (IL-2R) which is comprised of α, β and γ subunits [60]. Together IL-2 and IL-2R form a strong autocrine stimulatory loop for promoting T cell growth and clonal expansion. IL-2 also contributes to B cell differentiation and stimulates both macrophage and NK cell activation [151]. HMGA1 has been demonstrated to participate in the transcriptional activation of both the IL-2 [9,69,70] and IL-2α [76,76,77] genes in activated lymphoid cells. Furthermore, during the activation of both genes, HMGA1 has been implicated in the 'remodeling' or removal of inhibitory nucleosomes which, in unstimulated T cells, are positioned on the recognition sites of regulatory key factors that are required for transcription initiation.

Similar to induction of IFN-β, transcriptional activation of IL2-Rα is regulated by enhanceosome formation involving the interaction of HMGA1 with other transcription factors (Elf-1, STAT5, GATA, etc.) and several positive regulatory regions (e.g., PRRI, PRRII and PRRIII) in the gene's proximal promoter [76,77]. However, in contrast to the promoter of IFN-β, which in uninfected cells has two positioned nucleosomes flanking naked DNA where the enhanceosome forms, the promoter of the IL-2Rα in resting T cells has a strongly positioned nucleosome that encompasses one of the positive regulatory regions (i.e., PRRII), the site where an enhanceosome is assembled. The PRRII region contains two stretches of A/T-rich DNA, potential binding sites for HMGA1, one of which partially overlaps the recognition sequence for Elf-1, a transcription factor that is required for both enhanceosome formation and IL-2Rα gene activation *in vivo*. Following T cell activation the inhibitory nucleosome on the PRRII region is removed/remodeled, enhanceosome assembly ensues and transcription is initiated [137].

Chromatin reconstitution experiments employing IL-2Rα DNA have demonstrated that a nucleosome is assembled on the PRRII region *in vitro* at the same position as the one found *in vivo*. In this reconstituted nucleosome the A/T-rich stretch that overlaps the Elf-1 site is situated on the surface of the core particle and the second A/T-rich stretch is located in the

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

linker region immediately 5′ upstream of the nucleosome [137]. *In vitro* experiments using recombinant proteins showed that HMGA1, but not Elf-1, is able to bind the A/T-stretch on the surface of the nucleosome as well as to the stretch located in the adjacent linker region. Importantly, it was found that two different HMGA1 protein molecules bound to the two separate A/T-rich stretches in a direction-specific, "tail-to-tail" manner *in vitro*. If this *in vitro* pattern of HMGA1 binding likewise occurs *in vivo* it could potentially impart a unique identifying 'marker' or fingerprint to the positioned PRRII nucleosome that distinguishes it from all of the other core particles in the cell [137]. Based on these observations, and the fact that HMGA1 protein is present at only very low levels in resting lymphocytes but is rapidly induced in T cells following stimulation [64], a model for activation of the IL-2Rα promoter has been proposed [135]. In this model HMGA proteins are actively involved in both the initial disruption of the PRRII nucleosome (likely mediated via direct binding to the core particle and recruitment of remodeling factors) and in the subsequent formation of an enhanceosome on this same region of promoter DNA. The dual ability of HMGA proteins to bind to A/T-rich DNA sequences on the surface of nucleosome particles [137,141] and to also participate in enhanceosome formation, makes it is reasonable to expect that a similar activation scenario applies to other inducible genes whose promoters contain positioned nucleosomes and whose expression is controlled by HMGA proteins (e.g., the human IL-2 cytokine gene [9,69,70]).

Consistent with the above prediction, there are additional reports in which HMGA proteins have been demonstrated to facilitate recruitment of chromatin remodeling complexes to gene regulatory regions containing positioned nucleosomes during the transcriptional activation process. One particularly illuminating example involves the transcriptional activation of the latent immunodeficiency virus type 1 (HIV-1) provirus in human cells, a process that involves HMGA1 [64]. Following HIV-1 integration into the genome, the 5′ long terminal repeat (LTR) is packaged into an array of nucleosomes specifically positioned with respect to DNA sequence elements that regulate the transcriptional activity of the provirus. One of these nucleosomes, nuc-1, is positioned immediately 3′-downstream of the transcription start site and inhibits proviral expression. Interestingly, the DNA encompassed by nuc-1 contains two A/T-rich stretches that are strong HMGA1 binding sites, one located on the surface of the nucleosome and the other that is situated at the 3′ exit of DNA from the core particle and which overlaps an AP1-3/ATF-3 transcription factor binding site that extends into the linker region [64]. Agents, such as phorbol esters, that stimulate T cell activation not only rapidly induce HMGA1 expression [34,55,111], but also lead to rapid disruption of nuc-1 and stimulation of HIV-1 proviral transcription [64]. Experiments have demonstrated that following treatment of Jurkat T cells with phorbol myristate acetate the transcription factors ATF-3 and JunB, as well as BRG-1 (the ATPase subunit of the human SWI/SNF chromatin remodeling complex) are recruited to the 3′ boundary of nuc-1 whereupon the nucleosome is disrupted. Analysis of this recruitment process revealed that ATF-3 and BRG-1 exist together as a complex and that it is ATF-3 that is responsible for targeting the human SWI/SNF (hSWI/SNF) to the overlapping HMGA1/ATF-3 site at the edge of the core particle. Importantly, though, it was also demonstrated that HMGA1 proteins are required for recruitment of hSWI/SNF to the nuc-1 nucleosome [65].

Activation of the human alpha-B-crystallin (CRYAB) gene is another well documented example in which HMGA1 has been demonstrated to play a crucial role in recruiting both BRG-1 and AP-1 to a positioned nucleosome on the gene's promoter that is remodeled during transcriptional initiation. CRYAB is a small heat-shock protein that is expressed at high levels in vertebrate eyes and which has been implicated in many cellular processes including the phenotypic changes associated with lens cell differentiation. Transcriptional activation of CRYAB has been demonstrated to depend on either BRG-1 containing, or hBRM-associated factor (BAF) containing, SWI/SNF chromatin remodeling complexes [90]. Recently Zhao and his colleagues identified a 30 bp DNA element in the gene's promoter situated between −90

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

to −60 bp immediately upstream from the start site that is responsible for BRG-1 mediated activation of CRYAB transcription and whose action is chromatin dependent [44]. Point mutagenesis studies demonstrated that this BRG-1 response element contains an A/T-rich site for HMGA binding and a consensus recognition site for the AP-1 transcription factor. Ligation-mediated PCR (LM-PCR) analyses demonstrated that this response element was located at the edge of a positioned nucleosome in non-BRG-1 expressing cells *in vivo*. Electrophoretic mobility "supershift" analyses of extracts from BRG-1 expressing cells and chromatin immunoprecipitation (ChIP) analyses of CRYAB expressing cells demonstrated that HMGA1 binds to the response element both *in vitro* and *in vivo*. And, finally, cell transfection experiments employing small hairpin RNAs demonstrated that HMGA1 proteins are critically required for the maximal activation of the CRYAB promoter by BRG-1. Based on these data the authors concluded that HMGA1, a BRG-1 containing SWI/SNF chromatin remodeling complex, and the sequence-specific transcription factors AP-1 act together to regulate maximal expression of the CRYAB gene *in vivo*. They further suggest that the most likely function of the HMGA1 protein is to facilitate and assist remodeling of the inhibitory nucleosome by the BRG-1/SWI/SNF complex [44].

Available data thus indicate that there are at least two ways that HMGA proteins can induce localized changes in the chromatin structure of inducible gene promoters, both of which involve positioned nucleosomes that must be 'remodeled' before gene transcription can occur. The first mechanism is exemplified by the way in which transcription of the IFN- β gene is activated in virus infected cells. In this case HMGA1 binds to, and coordinates the formation of, an enhanceosome on 'naked' promoter DNA that is flanked on either side by two strongly positioned nucleosomes. In uninfected cells one of these nucleosomes is positioned over the TATA box region, thus inhibiting transcription. Once formed, the IFN-β enhanceosome recruits chromatin modifying and remodeling complexes, the later of which induces sliding of the inhibitory nucleosome and exposure of the TATA box so that TBP/TFIID can bind and PolII transcription can be initiated. The second mechanism is epitomized by the way in which the promoters of the IL-2, IL-Rα, CRYAB and the 5′ LTR of the HIV-1 virus, are activated. In each of these cases, prior to transcriptional activation a nucleosome is stably positioned on a regulatory DNA element that contains binding sites for transcription factors (e.g., HMGA1, Elf-1, AP-1, etc) that are required for enhanceosome formation. Hall marks of these positioned nucleosomes include: (i) the binding site for a sequence-recognizing transcription factor involved is occluded, thereby preventing its binding; (ii) there are one or more stretches of A/T-rich DNA position either on the surface of the nucleosome and/or adjacent to one of its edges; and, (iii) the binding sites for HMGA1 and the transcription factor involved frequently overlap and often extending into the adjacent linker region. The roles played by HMGA1 in the disruption of such inhibitory nucleosomes appear to include binding to the A/T-rich regions, recruitment of chromatin remodeling complexes and enhancement of their activity plus participation in enhanceosome formation on, or over-lapping, the original site of the positioned core particle.

V. HMGA Proteins and Global Chromatin Structure

In addition to affects on localized nucleosome structure it has long been appreciated that HMGA proteins also significantly influence larger scale chromatin domains and even the structure of whole chromosomes. For example, soon after their discovery in HeLa cell extracts [95] HMGA1 proteins were demonstrated to specifically bind to the highly repetitive A/T-rich alpha-satellite DNA found in monkey cells [153], suggesting that they might be integral components of chromatin structure. That this is indeed the case was subsequently confirmed by immunofluorescence staining studies demonstrating that HMGA proteins are localized to the A/T-rich G/Q- and C-bands of human and mouse metaphase chromosomes [43]. Their localization to specific regions of metaphase chromosomes led to the prediction that HMGA1

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

proteins are actively involved in the dynamic changes in chromatin structure that accompany the chromosome condensation and cell division cycles [43], a suggestion that is supported by a substantial body of evidence (reviewed in: [140]).

For example, it has been demonstrated that treatment of cells with either distamycin A or Hoechst 33258, minor groove binding chemicals that directly compete with the HMGA proteins for binding to AT-DNA *in vitro* [164], prevents complete condensation of metaphase chromosomes *in vivo* [129]. Likewise, HMGA1 proteins have been demonstrated to be *in vivo* substrates for cdc2 kinase which phosphorylates specific threonine residues located adjacent to two of the three AT-hooks of the protein during the G2/M-phase of the cell cycle. These phosphorylations reduce the binding affinity of the modified HMGA1 proteins for A/T-rich DNA by more that 20-fold [109,136] and are likely to be associated with the marked changes in chromosome structure and dynamic protein interactions that are occurring during the G2/M phase of the cell cycle. Additionally, these modifications may also be associated with the reversible shuttling of a subpopulation of HMGA1 proteins between the nucleus and mitochondria that occurs around this stage of the cell cycle in normal cells [40,98].

Indications that HMGA proteins are involved in regulating the structure and function of large chromatin domains, comes from the early work of Laemmli and his colleagues [143]. Employing immunolocalization techniques these workers demonstrated that HMGA1 proteins are co-localized with both histone H1 and the enzyme topoisomerase II at sites called scaffold attachment regions (i.e. SARs), A/T-rich DNA sequences that constitute the structural backbone of metaphase chromosomes. SARs are thought to be cis-acting DNA elements located at the base of large loops of gene-containing DNA and have been postulated to be involved in the dynamic regulation of both the chromatin structure and transcriptional activity the DNA loop or ‘domain’. Support for the idea that HMGA proteins participate in regulating the activity of such ‘domains’ comes from experiments with *in vitro* transcription systems developed to search for proteins capable of antagonizing histone H1-mediated repression of transcription from SAR containing plasmid DNA templates [178]. Employing such systems, HMGA1 was identified as the nuclear protein that was able to out-compete histone H1 for binding to SAR elements and, as a consequence, induce gene transcription from previously repressed plasmid templates. Based on these and other data, a model has been proposed in which H1 and HMGA1 proteins compete for binding to SAR elements located at the base of large chromatin domains with the winner of this competition determining both the condensation state of the domain and the transcriptional capacity of the genes it contains [178]. It was further suggested that such domain regulation was likely to transpire during embryonic development and other situations where large-scale alterations in chromatin structure and gene expression occur. Several lines of evidence support the HMGA1:H1competition model for determining the structure and activity of large chromatin domains. These include the fact that HMGA1 proteins are greatly enriched in, and histone H1-depleted from, the transcriptionally active subfraction of chromatin isolated from cells [178]. Additional support for the model comes from *in vitro* experiments demonstrating that artificial multi-AT-hook (MATH) proteins (synthetic mimics of HMGA proteins) that bind tightly to SAR sequences are potent inhibitors of chromosome condensation when added to mitotic extracts of *Xenopus* oocytes [154].

Apoptosis is another biological process in which HMGA proteins influence global chromatin structure. Apoptosis, or programmed cell death, is characterized by chronological alterations of mitochondrial and plasma membrane function followed by marked changes in nuclear morphology, chromatin condensation, DNA fragmentation and other dramatic changes in cellular phenotype [16,105]. But to understand the role HMGA proteins play in apoptosis, it is first necessary to consider the complex roles these proteins are thought to play in development and in cancer. Intracellular concentrations of HMGA proteins are maximal during embryogenesis but drop to very low, often nearly undetectable, levels in normal differentiated

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

somatic cells [27,179]. Most 'immortalized' pre-cancerous cells and nearly all overtly cancerous cells, on the other hand, constitutively over-express HMGA proteins with increasing concentrations being correlated with tumor progression, increasing metastatic potential and poor patient prognosis [58,131]. Over-expression of HMGA proteins in normal cells is toxic and induces apoptosis. Fedele *et al*. have demonstrated, for example, that forced over-expression of HMGA1b in normal rat thyroid epithelial cells does not transform cells but, rather, induces apoptosis as a result of deregulation of S phase DNA synthesis, delaying entry of cells into the G2/M phase of the cell cycle and activation of the caspase-3 death pathway [50]. The molecular mechanisms by which over-expression deregulates cell cycle progression are undoubtedly complex but include HMGA mediated aberrant induction of transcription of genes such as cyclin A [156] and AP-1 [162].

Once apoptosis has been initiated the HMGA proteins themselves undergo marked changes in both the types and extent of their post-translational modifications (PTMs; review in: [149, 175], some of which are likely correlated with alterations in chromatin structure. For example, the early stages of apoptosis are first accompanied by a global hyper-phosphorylation, which is quickly followed by a massive de-phosphorylation, of the total population of cellular HMGA proteins [41]. Since phosphorylation of HMGA proteins significantly reduces their binding affinity for A/T-rich DNA [109,136], hyper-phosphorylation likely results in partial displacement of HMGA proteins from DNA and the formation of a less condensed chromatin structure that is more easily digested by nucleases. Hypo-phosphorylated HMGA proteins, on the other hand, have an increased overall positive change that facilitates tighter DNA binding, chromatin condensation, and placement of HMGA:DNA complexes into apoptotic bodies [41]. Other site-specific PTMs of HMGA proteins, such as acetylation of K60 of HMGA1b [50] and methylation of R25 of HMGA1a [146–148], have also been reported to occur during apoptosis but the function(s) of such modifications in the death process are unknown.

In contrast to induction of apoptosis in normal cells, over-expression of HMGA proteins in some 'pre-disposed' normal cells, as well as in immortalized cell lines that have already breached the senescence barrier, often has anti-apoptotic affects [85–87]and frequently induces overt cancerous transformation [34,72,168,169]. A variety of mechanisms contribute to anti-apoptosis including: (i) binding of HMGA to HIPK2 kinase, a proapoptotic activator of p53, and chaperoning its relocalization from the nucleus to the cytoplasm [121]; (ii) binding of HMGA to p53 and interfering with p53-mediated transcription of apoptotic and cell-cycle arrest effectors [54,120]; and, (iii) cooperation of HMGA with other factors in the transcriptional activation of the p53 inhibitor MDM2 [120]. HMGA1 also regulates the transcriptional expression of a number of genes coding for micro-RNAs (miRNAs) [39], some of which (e.g., miR-29) could potentially interfere with apoptosis [83,101].

HMGA proteins have recently been directly implicated in the process of senescence, or aging, of normal cells. During senescence, a state of irreversible cell growth arrest, chromatin structure undergoes intricate and programmed global changes and these nuclear alterations are accompanied by equally complex alterations in the phenotypes of both aging cells and organisms [118]. A variety of molecular mechanisms can trigger senescence including DNA damage, acquisition of an active oncogene, telomere shortening and derepression of the INK4a/ARF locus and activation of the retinoblastoma (pRB) tumor suppressor pathways (reviewed in: [36,113]). Together these mechanisms limit excessive or aberrant cellular proliferation and, as a consequence, senescence is thought to both protect organisms against the development of cancer and to be a component of aging [35,75,112]. Senescence is accompanied by marked changes in the epigenetic state of chromatin and by widespread nuclear heterochromatin formation [4,11,108,142,145]. For example, during senescence of human primary fibroblasts (e.g., WI38 and IMR90) chromosomes undergo profound condensation with each chromosome forming a punctuate heterochromatic domain called a senescence-associated heterochromatic

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

focus (SAHF) [56,176]. The core histones of SAHFs are hypoacetylated due to increased levels of histone deacetylases (HDACs) in these structures [10]. Interestingly, the histones in SAHFs do not exhibit the typical PTM patterns that mark core histones of condensed mitotic chromosomes, apoptotic chromatin, or transcriptionally inactive heterochromatin [56]. SAHFs do, however, contain histone H3 that is methylated on lysine 9 (H3K9Me), a modification that provides a docking site for repressive polycomb proteins which are known markers for constitutive heterochromatin. SAHFs also contain heterochromatin protein 1 (HP1) and the transcriptionally repressive histone variant macroH2A [56,106,176]. And, importantly for the present discussion, SAHFs are depleted in linker histone H1 and enriched for HMGA1 and HMGA2, proteins which have been demonstrated to be essential components of these heterochromatin structures [56,107,176].

Formation of SAHFs in human cells is a dynamic and multifaceted process that is largely dependent on the p16/pRB *ras*-induced senescence pathway [108]. Many details remain to be elucidated but formation appears to be driven by a complex of histone chaperones (HIRA and ASF1a), a pRB/hBrm/HDAC1/HP1β chromatin remodeling complex plus an additional chaperone protein (HIRA-3) that physically links these two complexes together and coordinates their activity [4,106,177]. It has been proposed that the hBrm component of the remodeling complex is required for incorporation of histone variants such as macroH2A in SAHFs [10]. It is not know whether the hBrm-containing complex, the HIRA-containing complex or another yet unidentified complex, contributes to the recruitment of HMGA proteins to SAHFs. What has been demonstrated, however, is that incorporation of HMGA proteins into SAHFs is linked to simultaneous displacement of histone H1 [56]. As previously mentioned, HMGA proteins can compete histone H1 for binding to a variety of different types of DNA substrates [66,68,178]. It has been suggested, therefore, that SAHF formation is a novel type of chromatin condensation involving alterations in linker DNA-binding proteins with HMGA out-competing and replacing histone H1 [56]. It should be noted that this model for SAHF formation is very similar to the previously discussed model proposed by Laemmli's group [78,82,178] except that, in this case, HMGA displacement of histone H1 leads to chromosome condensation rather than to chromatin domain de-condensation and 'opening'. Since HMGAs are among the most highly modified proteins in the nucleus and such PTMs influence their substrate binding characteristics (review in: [135,175], these two different effects of HMGA proteins on chromatin structure could well be due to differences in the types of biochemical modifications present on the proteins in SAHFs and in activated chromatin domains.

In light of the fact that over-expression of *HMGA* genes causes neoplastic transformation of pre-disposed cells *in vitro* and also induces tumors in transgenic mice *in vivo* [171] [49,51], the specific accumulation of HMGAs on chromatin during senescence and a requirement for these proteins in SAHF formation were initially perplexing [107]. Although there is still no completely satisfactory answer to this apparent paradox, available data derived from experiments involving knock-down and knock-in of various SAHF components in transfected cells, combined with the use of drugs that compete for HMGA binding to the minor groove A/T-rich DNA (summarized in [106]), have suggested that HMGA contributes to SAHF formation by modulating higher-order chromatin structure and limiting its accessibility to transcription factors. Thus, just as in the case of apoptosis, HMGA proteins can be either pro- or anti-oncogenic, depending on the cellular context and the biochemical modification state of the proteins. Consistent with this ying-yang view, it has been reported that malignant haematologic tumors develop in both transgenic mice that over-expressing HMGA1 [49] and in *Hmga1* knock-out mice that are devoid of this protein [52].

HMGA proteins also influences the capacity of cells to repair damaged DNA by affecting chromatin structure at both the genome-wide and gene-specific levels [97,134]. HMGA over-

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

expression sensitizes cancerous cells to killing by various genotoxic agents such as UV-light, cisplatin, hydrogen peroxide, menadione and methymethanesulfonate (MMS) [2,98,134]. In the case of UV-light, which induces backbone-distorting cyclobutane pyrimidine dimers (CPDs) and 6-4 pyrimdine-pyrimidone (6-4-PP) photoproducts into DNA, the increased killing has been causally linked to inhibition of the nucleotide excision repair (NER) pathway that removes bulky lesions from damaged DNA [2,3,97]. This repair inhibition is mediated by: (i) binding of HMGA protein directly to CPD and 6-4PP lesions and preventing access of NER repair proteins by steric hindrance [2]; and, by: (ii) specific binding of HMGA to the promoter region of *XPA*, a gene whose protein product is essential for efficient NER, and inhibiting its transcription [2]. It seems likely that HMGA over-expression also inhibits repair of non-bulky lesions that are removed by the base excision repair (BER) pathway by similar mechanisms [98,134].

VI. HMGB Proteins and Chromatin Structure

The multifaceted roles played by HMGB (formerly called HMG-1 and -2) proteins in modulating chromatin structure, gene transcriptional activity and cellular phenotype have been covered in a number of recent reviews and readers are referred to these for in-depth coverage of these topics [6,14–17]. Here emphasis is placed on comparing the similarities and differences of these proteins with the other HMG families. The mammalian HMGB proteins are characterized by two tandem DNA-binding regions called HMG box domains followed by a 30 amino acid long acidic tail. Each of the approximately 80 amino acid long HMG box domains has a similar structure which is composed of three α-helices folded into an L-shaped configuration whose concave surface binds into the minor groove of DNA with limited or no sequence specificity [158]. The motion and DNA binding activity of the two HMG boxes are independent of each other and the acidic tail is unstructured [79], characteristics that, as is the case with HMGA proteins, impart considerable structural and substrate recognition flexibility to the proteins. The HMG box domain is an ancient structural motif that is present in one or more copies in non-HMG proteins in eukaryotic organisms ranging from yeast, to sponges to plants [15].

HMG boxes bind to the minor groove of B-form DNA, significantly widen the groove and introducing a bend of 90° or more into the backbone. HMGB proteins also bind with high affinity to already distorted DNA structures such as four-way junctions, bulges, kinks and modified DNA containing cisplatin adducts [122]. The induction of DNA bends and the recognition of distorted DNA structures are the two main ways that HMGBs function as chromatin ‘architectural’ proteins. For example, it has been suggested that HMGB-induced DNA bending produces an allosteric transition structure that promotes the recognition and binding by other proteins during the formation of functional multiprotein:DNA complexes. On the other hand, HMGB recognition and binding to already distorted DNA conformations is thought to be analogous to enzymes recognizing molecular structures that resemble transition states between reagents and products and, as a consequence, influencing the rate of formation of multiprotein:DNA complexes [6].

HMGB proteins participate in, and facilitate, many different nuclear processes including transcription, replication, V(D)J recombination, DNA repair and other activities. HMGB proteins promote the transcription of genes through several mechanisms [6,15]. One mechanism is mediated by the ability of HMGB proteins to bind to nucleosomes [159]. HMGB can bind to DNA segments at the entry/exit of nucleosomes (whose 3D-structure has been suggested to resemble a four-way junction [180]) in much the same way histone H1 [26]. However, in contrast to H1 binding, which is thought to lock nucleosomes in place and make them less mobile and less accessible to transcription factors [26], HMGB binding facilitates

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

recruitment of chromatin remodeling proteins (e.g., ACF/CHRAC) that induce nucleosome sliding, thus exposing previously blocked regions of DNA [20].

A second mechanism, proposed on the basis of results from EMSA competition and other *in vitro* assays, involves HMGB in a context where it acts as a transcriptional repressor, binding to the TATA-binding protein (TBP) to form a stable HMG-1/TBP/TATA complex, which is proposed to inhibit the assembly of the preinitiation complex on gene promoters [37]. Additional experiments have demonstrated that the accessory transcription factor TFIIA can also bind to TBP and displace HMGB1 from the inhibitory HMGB1/TBP/TATA complex, thus allowing a stable preinitiation complex to form and promote the early stages of transcriptional initiation [38]. The generality of this proposed inhibitory role of HMGB in transcriptional initiation remains to be determined.

A third, and much better characterized mechanism, is the so called ‘hit-and-run’ mode of action in which HMGB proteins facilitate the stable binding of other transcription factors to their DNA recognition sites but then dissociates from any ternary complex that is formed leaving the partner protein stably bound to its DNA substrate (reviewed in [6]). In these cases HMGB is thought to function as a transient protein chaperone that mediates a ‘match’ and then leaves the couple alone after their union. HMGB proteins are know to make weak, but specific physical interactions with a number of different transcription factors including p53, all class I steroid receptors, TBP, RAG1, HOX and POU proteins, several NF-κB subunits and others. A number of different types of hit-and-run pathways involving HMGB and these transiently associating proteins have been reported but generally appear to fall into three distinct categories [15]. One pathway is exemplified by p53 which binds only very weakly to its recognition site in B-form linear DNA. In this case, HMGB first binds and bends the DNA, presents it to p53 which then binds tightly to its pre-bent recognition sequence and HMGB leaves the complex. A second pathway involves proteins such as RAG1 and the glucocorticoid receptor (GR). These proteins also bind only weakly to their recognition sequences in linear DNA but, as a result of binding introduce a slight distortion in their target sites. HMGB recognizes and binds to these distorted structures with high affinity, thus stabilizing and strengthening the interaction of the partner protein (e.g., RAG1 or GR) to DNA and then HMGB leaves the complex. Interestingly, in the case of the glucocorticoid receptor it has been demonstrated that these HMGB-GR interactions can only occur within the context of chromatin [7]. In a third pathway, HMGB interacts with its partner protein prior to their actual binding to DNA, a coordinated DNA binding/bending occurs following protein:DNA complex formation, straight away followed by exit of HMGB from the complex.

Weak and transient interactions of HMGB proteins in such hit-and-run mechanisms are understandable given that ‘fluorescence loss in photobleaching’ (FLIP) experiments employing GFP-labeled HMGBs have shown that in living cells they are the most mobile of all nuclear proteins [144]. The entire pool of HMGB1 has been demonstrated to rapidly diffuse throughout the entire nucleus with each individual nucleosome being visited by HMGB1 approximately every 2 seconds and then dwelling there for only a fraction of a second. Thus, each HMGB-protein interaction is weak and transient. Nevertheless, since the intra-nuclear concentration of HMGB1 is in the micromolar range, at any given moment a significant fraction of the partner protein is engaged in interactions with HMGB1 simply because its concentration is so high [6].

Not all functional interactions of HMGB proteins are of the hit-and-run variety, however. In the case of the BHLF-gene, HMGB facilitates the formation of an enhanceosome on its promoter and remains a stable part of the complex following transcriptional activation [46]. It has also been demonstrated that HMGB proteins bind with high affinity to cisplatin adducts and shield these DNA-distorting lesions from repair processes [63], another example where

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

HMGB proteins apparently form part of a stable DNA complex *in vivo*. It should also be noted, however, that binding of HMGB to some DNA lesions does not result in inhibition of repair. For example, when certain genotoxic nucleoside analogs (e.g., deoxythioguanosine) are incorporated into DNA, HMGBs target a complex consisting of HMGB1 and -2, HSC70, Erp60 and the cytoplasmic protein GAPDH to the mis-incorporated base pair and stimulate its removal [81]. Thus, depending on the lesion, HMGB proteins can either inhibit or facilitate DNA repair processes.

But perhaps the most remarkable example of stable HMGB1 complex formation *in vivo* occurs during apoptosis when the usual dynamic movement of this protein is completely arrested and nuclear chromatin undergoes condensation and fragmentation on a global scale [144]. This stable complex formation seems to be specific for HMGB1 since other nuclear proteins do not show a similar reduction in mobility during chromatin condensation. Since the biochemical modification state of HMGB1 doesn't change during apoptosis, the basis for stable binding likely resides in the condensed apoptotic heterochromatin itself. The signal for such binding is unknown but it has been suggested that it might be due to either HMGB recognition of (and binding to) the hypoacetylated N-terminal histone tails found in heterochromatin or to some structural change in chromatin resulting from the large-scale histone deacetylation that occurs during heterochromatin formation [6].

The apparent biological function of tight binding of HMGB to chromatin in apoptotic cells is to prevent the protein from leaking out of dying cells and triggering an inflammatory response [16]. HMGB (also known as amphoterin and sulfoglucuronyl carbohydrate binding protein (SBP-1) in the field of immunology) leaks out from necrotic cells as an ‘alarmin’ molecule that signals to neighboring cells that tissue damage has occurred and induces them to divide, migrate, activate inflammation or start an immune response. HMGB is also naturally secreted from monocytes, macrophages, neurtrophils and other immune cells following exposure to LPS, IL-1β, TNF-α, or other stimulatory agents, and is a late mediator of sepsis [163]. Discussion of this facinating ‘alter ego’ aspect of HMGB function is, however, beyond the scope of this article and readers are referred to a recent review by Bianchi and Manfreidi [17] for coverage of this topic. Nevertheless, an important point to make concerning the immune functions of HMGB is that post-translational modifications regulate its nucleocytoplasmic shuttling and its secretion from cells. For example, phosphorylation regulates its nucleocytoplasmic shuttling and re-directs it towards secretion [174] and methylation on specific residues causes cytoplasmic retention of HMGB in nucleophils [74].

It is now thought that tight binding of HMGB to condensed chromatin may not be restricted to apoptotic cells. That HMGB proteins might actually be involved in the organization and/or maintenance of heterochromatic regions is suggested by the results of experiments analyzing the stable binding of the HMGB2 protein, in complex with nucleolin and YY1 proteins, to the heterochromatic DZ4Z tandem repeat sequences in the subtelomeric region of human 4q35 [59]. Thus, like the HMGA proteins, the HMGB proteins can impact the structure of chromatin at both the local nucleosomal level and on larger, much more extensive scales.

VII. HMGN Proteins and Chromatin Structure

The five principal members of the HMGN (formerly called HMG-14 and -17) protein family (HMGN1, HMGN2, HMGN3a, HMGN3b and HMGN4) are found only in vertebrates [24]. Detailed studies of developing vertebrate embryos show that the levels of expression HMGN proteins are tightly linked to cellular differentiation [57,80]. For example, during mouse embryogenesis the level of expression of the two major protein variants, HMGN1 and HMGN2, is high in all tissues but progressively drops throughout the entire embryo as development and cellular differentiation proceed. Such a developmental drop in HMGN levels does not occur,

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

however, in continuously renewing cell types such as the basal kidney epithelium where cell proliferation and differentiation are ongoing processes [57,84]. Together these observations suggest that during embryonic development there is an inverse correlation between the level of HMGN proteins and the differentiation state of cells. This idea is supported by experiments with tissue culture cells which show that as cells differentiate their levels of HMGN drop [57,80] and that differentiation is inhibited when HMGNs are artificially over-expressed in cells [57,117]. Thus, proper cellular differentiation requires carefully regulated levels of HMGN expression. That it is the level, rather that the specific type, of HMGN proteins that is important for regulating differentiation is supported by the fact that homozygous *Hmgn1*$^{-/-}$ knock-out mice are viable but exhibit various subtle abnormalities (see below), suggesting that during embryogenesis HMGN2 is able to partially compensated for the loss of HMGN1, probably because of a limited functional redundancy of these very similar proteins [19].

HMGN proteins primarily function by promoting the unfolding of higher-order-chromatin structure and, thereby, enhancing transcription, replication and DNA repair from chromatin templates [24]. One of the principal mechanisms facilitating such unfolding is mediated by the ability of HMGN proteins to out-compete histone H1 for chromatin binding sites [29]. HMGN proteins are highly mobile within chromatin: nevertheless, more than 99% of HMGN1 molecules are bound to chromatin at any given moment, with residence-times ranging from 4 to 25 seconds, thus allowing for a dynamic competition to exist between HMGN and H1 proteins *in vivo* [119]. The precise mechanism by which HMGN proteins mediate chromatin unfolding remains unclear, however, since ATP-dependent chromatin remodeling complexes are apparently not involved, at least not *in vitro* [67,130]. Another mechanism contributing to HMGN-induced structural changes in chromatin is the ability of these proteins to affect the levels of post-translational modification in the tails of histone proteins [88,89,123,161]. Members of the HMGN family bind exclusively and tightly to nucleosome core particles and stabilize their structure [27]. HMGNs are also the only non-histone proteins known to bind specifically inside the nucleosome between the gyres of DNA and the histone octamer core with the tail of histone H3 and histone H2B being involved in the interaction [24].

All HMGN proteins contain three important functional regions: a bipartite nuclear localization signal (NLS), a 30 amino acid-long nucleosomal binding domain (NBD) and an acidic tail called the chromatin-unfolding domain (CHUD) [24]. Unlike the substrate binding domains of the other HMG families, the structure of the NBD of HMGN proteins has yet to be determined. Within the nucleus of interphase cells HMGN1 and HMGN2 proteins are distributed in distinct foci, consistent with the demonstration that stretches of contiguous nucleosomes contain only one type of HMGN homodimer, either HMGN1 or HMGN2, and never mixtures of HMGN1/2 proteins [124,125]. In metaphase cells, however, the NBD of the HMGN proteins is inactivated and the proteins associate with chromatin only as monomers with low binding affinity. Neither do HMGN proteins form specific complexes in metaphase cells, demonstrating that the mode of binding of HMGNs to chromatin is cell cycle dependent [32].

HMGN proteins are subject to extensive post-translational modifications which influence both their mode of binding to chromatin and their functional activity. Like the other HMG protein families, HMGNs are substrates for many of the same enzymes that modify histone proteins (review in: [175]). The histone acetyltransferase (HAT) enzyme p300, for example, acetylates both HMGN1 and HMGN2 whereas the HAT enzyme pCAF (p300/CBP-associated factor) acetylates only HMGN2. It has been demonstrated that acetylated HMGNs bind nucleosomes less tightly than un-modified proteins and, therefore, exhibit a decreased ability to unfold compact chromatin [24]. Likewise, phosphorylation decreases the interaction of HMGN proteins with nucleosomes [114]. Cell cycle-dependent phosphorylation of serine residues on HMGN1 (at S6, S20 and S24) specifically prevent it from binding to mitotic chromosome,

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

increase its mobility inside the nucleus, inhibits its nuclear import and promotes its interaction with cytoplasmic 14.3.3 proteins [127,128]. Several protein kinases have been demonstrated to phosphorylate HMGNs, including PKC, PKA, CK2, GMP-dependent protein kinase and mitogen-and stress-activated kinases (MSKs) [175]. MSKs are the major kinases responsible for phosphorylating HMGN1 (at S6) and histone H3 (at S10) in what is called the 'nucleosome response' which leads to the transcriptional activation of immediate early (IE) genes in mouse fibroblasts exposed to mitogenic or stress stimuli [152]. Phosphorylation of HMGN1 leads to a transient weakening of it's binding to chromatin and allows kinases to subsequently access and phosphorylate the N-terminal tail of histone H3. Since phosphorylation of HMGN1 precedes that of H3, it has been suggested that HMGN1 plays an important role in modulating the 'histone code' [88].

Numerous i*n vitro* studies have demonstrated that HMGN1 proteins enhance transcription in the context of chromatin, suggesting that it acts as a genome-wide transcriptional coactivator [42,116,160]. Analysis of embryonic fibroblasts from *Hmgn1*$^{-/-}$ mice, however, have shown that loss of HMGN1 leads to both up and down-regulation of gene expression [18,19], suggesting that specific subsets of genes may be differentially regulated by different HMGN proteins. Available evidence supports this idea but does not rule out the possibility that HMGN proteins can also have gene specific activities. For example it has been demonstrated that stable expression of HMGN3a and HMGN3b proteins in cells (Hepa-1) that contain no endogenous HMGN3 induces the up-regulation of a small subset of only 22 genes, one of which is the glycine transporter, *Glyt1* [166]. Likewise, experiments with embryonic fibroblasts from *Hmgn1*$^{+/+}$ and *Hmgn1*$^{-/-}$ mice examining the effect of HMGN1 on the heat shock-induced transcriptional activation of the *Hsp70* gene demonstrated that HMGN1 specifically enhances the rate of heat shock-induced H3K14 acetylation in the *Hsp70* promoter, thereby enhancing the rate of chromatin remodeling and the subsequent transcription during the early rounds of *Hsp70* transcriptional activation [13].

Homozygous knock-out *Hmgn1*$^{-/-}$ mice are viable but subfertile, exhibit minor developmental abnormalities and are hypersensitive to various stress conditions, such as exposure to UV light or ionizing irradiation [18]. The incidence of spontaneous tumors in *Hmgn1*$^{-/-}$ mice is also almost twice that of wild-type mice and cells derived from *Hmgn1*$^{-/-}$ mice have an increased tumorigenic potential, as measured by colony formation in soft agar and generation of tumors in nude mice [18]. Following UV irradiation, the transcription profile of *Hmgn1*$^{-/-}$ cells is altered but expression of the genes in the NER pathway is normal [19]. When *Hmgn1*$^{-/-}$ cells are stably transfected with a plasmid expressing wild-type HMGN1 protein (but not with a plasmid expressing HMGN1 lacking the CHUD domain) the reconstituted cells are restored to a wild-type phenotype with respect to UV sensitivity and turmorigenic potential. Together these findings indicate that HMGN1 enhances the rate of repair of UV-induced lesions by decompacting chromatin and facilitating the access of NER proteins to the damaged sites [19,165].

Additional support for a role of HMGN1 in enhancing DNA repair comes from studies of transcription-coupled-repair (TCR) in human cells. Lesions in the template strand of DNA induced by UV light stall the movement of RNA polymerase II (RNAPII) and interrupt transcription. Removal of these transcription-blocking lesions by TCR is required for the resumption transcription elongation. In mammals, TCR is dependent on Cockayne syndrome A and B (CSA and CSB) proteins. Recent investigations by Mullenders and his colleagues [53] have demonstrated that CSA and CSB play different roles in recruiting TRC-specific factors to UV-stalled RNAPII during repair complex formation. The CSB protein plays an essential role in lesion repair by acting as a coupling factor that attracts other repair factors (including HAT p300, NER proteins and a ubiquitin ligase/signalosome complex of proteins containing CSA) to the stalled RNAPII. Interestingly, CSA is not necessary for attraction of

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NER proteins to lesion- stalled RNAPII and yet, in cooperation with CSB, is required to recruit HMGN1 and certain transcriptions factors to the repair complex. Thus, recruitment of HMGN1 to stalled RNAPII complex is a relatively late event during TCR and likely plays a role in altering chromatin structure to facilitate lesion removal and the re-initiation of transcription [53].

VIII. Conclusions: Three's Company

Fluorescence recovery after photobleaching (FRAP) techniques have demonstrated that in living cells the interaction of chromatin binding proteins with their nuclear targets is dynamic and transient, with many of the proteins diffusing through the entire nucleus on a time scale of seconds [119]. Photobleaching experiments have also shown that there are highly dynamic, three-dimensional networks of protein-chromatin interactions in the nucleus that influence chromatin structure on a global scale [119]. Employing cell microinjection and FRAP techniques, Catez *et al* [31] investigated whether members of the different HMG families could compete with histone H1 for binding to chromatin substrates inside cells, as would be predicted from the results of numerous *in vitro* experiments. Purified recombinant proteins from each of the three HMG families (HMGA, HMGB and HMGN) were microinjected into the cytoplasm of cells expressing transgenic histone H1-green fluorescent protein (H1-GFP) and FRAP was used to compare the mobility of H1-GFP in injected cells to that in uninjected control cells. The results showed that proteins from each of the three HMG families weakened the binding of H1 to nucleosomes by dynamically competing for chromatin binding sites *in vivo*. Furthermore, it was found that the different HMG families do not compete with each other for chromatin binding. Rather, the HMG families act synergistically to weaken H1 binding to chromatin, results that suggest each HMG type competes with H1 for unique binding sites and for the formation of distinct protein complexes around different nucleosome linker DNAs. Based on these findings, Catez *et al*. proposed that a network of dynamic and competitive interactions involving HMG proteins and H1, and perhaps other structural proteins, constantly modulates nucleosome accessibility and the local structure of the chromatin fiber. As a consequence of such competition, H1 displacement by HMGs loosens up the higher-order structure of the chromatin fiber thereby allowing a 'window of opportunity' for regulatory factors to access previously restricted regions and to modulate gene transcription [31].

The independent nature of chromatin binding and competition exhibited by each of the different HMG families provides a possible raison d'être for the existence of three groups of proteins that share many over-lapping characteristics and functions, but with each group retaining its own distinguishing features and activities. The conceptual and mechanistic problems surrounding the issue of how to precisely regulate the structure of nuclear chromatin, with its myriad of dynamic subdomains that are constantly changing in response to cell cycle, developmental, environmental signals, are immense. A network in which three different families of HMG proteins, each of which binds to chromatin in a distinctive way and counteracts the condensing affects of histone H1, likely provides a degree of dynamic flexibility to chromatin structure that would not be possible if only a single family, or even two families, existed. A triumvirate of architectural transcription factors acting together to modulating chromatin structure also constitutes a powerful mechanism for the fine-tuning of gene transcription during embryonic development or in response to rapid changes in the environment. Such a network, in which members within each family can (if required) partially compensate for each other, also provides the cell with a 'buffering capacity' and degree of biological resilience that probably could not be achieved with fewer components.

It is instructive to consider, in general terms, both the over-lapping and distinctive features of the different HMG families with respect to various nuclear and biological processes. All three HMG families, but not necessarily the individual members of each family, have been

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

demonstrated to play critical roles in normal embryonic development and cellular differentiation. Likewise, all three families actively participate in transcription, replication and DNA repair, but do not always perform the same function(s) in these different processes. Of the three families, the function of the HMGN proteins in these processes is easiest to envision. HMGN proteins are positive regulators of transcription, enhance repair of DNA lesions and participate in the dynamics of chromatin replication during the cell cycle. Their primary mode of action in all of these events is to facilitate the loosening of the higher-order structure of the chromatin fiber, either by displacing histone H1 and/or by influencing the secondary modifications on the core histones, thus allowing access of the factors required to complete these processes. Post-translational modifications play important roles in regulating the function of the HMGN proteins, particularly during progression through the mitotic cycle.

The roles of the HMGA and HMGB proteins in these same biological events are much more complex and are often yin-yang in character. Depending on the cell type and the specific circumstances, the HMGA and HMGB families can function as either positive or negative regulators of gene transcription, chromatin replication and, in the case of HMGB proteins, DNA repair. The HMGA and HMGB proteins can act at both the individual nucleosome- and at the global chromatin domain-levels to influence these processes by, for example, promoting, or inhibiting, the recruitment of chromatin modifying and remodeling complexes. As with the HMGN proteins, post-transcriptional modifications of the HMGA and HMGB proteins are causally associated with how they function in the cells. The impact of secondary modifications on the function of HMGA and HMGB proteins is particularly evident during events such as apoptosis, necrosis and senescence, a significant group of biological processes in which the HMGN family apparently does not participate.

Given the plethora of chromatin states and physiological processes in which the HMG proteins are active participants, it is somewhat surprising that there aren't more architectural transcription factor families. Nevertheless, either through parsimony or simply by chance, evolution has established that only three families of HMG proteins are 'sufficient' for all of the nuclear activities required for the development and survival of mammals. Elucidating the mechanistic details of how even this limited number of protein families fulfills their vital functions will, however, remain the focus of intense research for many years to come.

Acknowledgments

This work was supported, in part, by NIH grant R01-GM071760.

X. References and citations

1. The HMG Chromosomal Proteins. Academic Press; London: 1982.
2. Adair JE, Kwon Y, Dement GA, Smerdon MJ, Reeves R. Inhibition of nucleotide excision repair by high mobility group protein HMGA1. J Biol Chem 2005;280:32184. [PubMed: 16033759]
3. Adair JE, Maloney SC, Dement GA, Wertzler KJ, Smerdon MJ, Reeves R. High-mobility group A1 proteins inhibit expression of nucleotide excision repair factor xeroderma pigmentosum group A. Cancer Res 2007;67:6044. [PubMed: 17616660]
4. Adams PD. Remodeling chromatin for senescence. Aging Cell 2007;6:425. [PubMed: 17635419]
5. Agalioti T, Lomvardas S, Parekh B, Yie J, Maniatis T, Thanos D. Ordered recruitment of chromatin modifying and general transcription factors to the IFN-beta promoter. Cell 2000;103:667. [PubMed: 11106736]
6. Agresti A, Bianchi ME. HMGB proteins and gene expression. Curr Opin Genet Dev 2003;13:170. [PubMed: 12672494]
7. Agresti A, Scaffidi P, Riva A, Caiolfa VR, Bianchi ME. GR and HMGB1 interact only within chromatin and influence each other's residence time. Mol Cell 2005;18:109. [PubMed: 15808513]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

8. Aravind L, Landsman D. AT-hook motifs identified in a wide variety of DNA-binding proteins. Nucleic Acids Res 1998;26:4413. [PubMed: 9742243]
9. Attema JL, Reeves R, Murray V, Levichkin I, Temple MD, Tremethick DJ, Shannon MF. The human IL-2 gene promoter can assemble a positioned nucleosome that becomes remodeled upon T cell activation. J Immunol 2002;169:2466. [PubMed: 12193716]
10. Bandyopadhyay D, Curry JL, Lin Q, Richards HW, Chen D, Hornsby PJ, Timchenko NA, Medrano EE. Dynamic assembly of chromatin complexes during cellular senescence: implications for the growth arrest of human melanocytic nevi. Aging Cell 2007;6:577. [PubMed: 17578512]
11. Bandyopadhyay D, Medrano EE. The emerging role of epigenetics in cellular and organismal aging. Exp Gerontol 2003;38:1299. [PubMed: 14698809]
12. Banks GC, Mohr B, Reeves R. The HMG-I(Y) A.T-hook peptide motif confers DNA-binding specificity to a structured chimeric protein. J Biol Chem 1999;274:16536. [PubMed: 10347218]
13. Belova GI, Postnikov YV, Furusawa T, Birger Y, Bustin M. Chromosomal protein HMGN1 enhances the heat shock-induced remodeling of Hsp70 chromatin. J Biol Chem 2008;283:8080. [PubMed: 18218636]
14. Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. J Leukoc Biol 2007;81:1. [PubMed: 17032697]
15. Bianchi ME, Agresti A. HMG proteins: dynamic players in gene regulation and differentiation. Curr Opin Genet Dev 2005;15:496. [PubMed: 16102963]
16. Bianchi ME, Manfredi A. Chromatin and cell death. Biochim Biophys Acta 2004;1677:181. [PubMed: 15020058]
17. Bianchi ME, Manfredi AA. High-mobility group box 1 (HMGB1) protein at the crossroads between innate and adaptive immunity. Immunol Rev 2007;220:35. [PubMed: 17979838]
18. Birger Y, Catez F, Furusawa T, Lim JH, Prymakowska-Bosak M, West KL, Postnikov YV, Haines DC, Bustin M. Increased tumorigenicity and sensitivity to ionizing radiation upon loss of chromosomal protein HMGN1. Cancer Res 2005;65:6711. [PubMed: 16061652]
19. Birger Y, West KL, Postnikov YV, Lim JH, Furusawa T, Wagner JP, Laufer CS, Kraemer KH, Bustin M. Chromosomal protein HMGN1 enhances the rate of DNA repair in chromatin. EMBO J 2003;22:1665. [PubMed: 12660172]
20. Bonaldi T, Langst G, Strohner R, Becker PB, Bianchi ME. The DNA chaperone HMGB1 facilitates ACF/CHRAC-dependent nucleosome sliding. EMBO J 2002;21:6865. [PubMed: 12486007]
21. Bourachot B, Yaniv M, Muchardt C. The activity of mammalian brm/SNF2alpha is dependent on a high-mobility-group protein I/Y-like DNA binding domain. Mol Cell Biol 1999;19:3931. [PubMed: 10330133]
22. Bustin M. Revised nomenclature for high mobility group (HMG) chromosomal proteins. Trends Biochem Sci 2001;26:152. [PubMed: 11246012]
23. Bustin M. At the crossroads of necrosis and apoptosis: signaling to multiple cellular targets by HMGB1. Sci STKE 2002;2002:E39.
24. Bustin M. Chromatin unfolding and activation by HMGN(*) chromosomal proteins. Trends Biochem Sci 2001;26:431. [PubMed: 11440855]
25. Bustin M. Regulation of DNA-dependent activities by the functional motifs of the high-mobility-group chromosomal proteins. Mol Cell Biol 1999;19:5237. [PubMed: 10409715]
26. Bustin M, Catez F, Lim JH. The dynamics of histone H1 function in chromatin. Mol Cell 2005;17:617. [PubMed: 15749012]
27. Bustin M, Reeves R. HMG Chromosomal Proteins: Architectural Components That Facilitate Chromatin Function. Prog Nucl Acids Res Molec Biol 1996;54:35–100.
28. Cairns BR, Schlichter A, Erdjument-Bromage H, Tempst P, Kornberg RD, Winston F. Two functionally distinct forms of the RSC nucleosome-remodeling complex, containing essential AT hook, BAH, and bromodomains. Mol Cell 1999;4:715. [PubMed: 10619019]
29. Catez F, Brown DT, Misteli T, Bustin M. Competition between histone H1 and HMGN proteins for chromatin binding sites. EMBO Rep 2002;3:760. [PubMed: 12151335]
30. Catez F, Lim JH, Hock R, Postnikov YV, Bustin M. HMGN dynamics and chromatin function. Biochem Cell Biol 2003;81:113. [PubMed: 12897844]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

31. Catez F, Yang H, Tracey KJ, Reeves R, Misteli T, Bustin M. Network of dynamic interactions between histone H1 and high-mobility-group proteins in chromatin. Mol Cell Biol 2004;24:4321. [PubMed: 15121851]
32. Cherukuri S, Hock R, Ueda T, Catez F, Rochman M, Bustin M. Cell cycle-dependent binding of HMGN proteins to chromatin. Mol Biol Cell 2008;19:1816. [PubMed: 18287527]
33. Cleynen I, d Van V. The HMGA proteins: a myriad of functions (Review). Int J Oncol 2008;32:289. [PubMed: 18202751]
34. Cmarik JL, Li Y, Ogram SA, Min H, Reeves R, Colburn NH. Tumor promoter induces high mobility group HMG-Y protein expression in transformation-sensitive but not -resistant cells. Oncogene 1998;16:3387. [PubMed: 9692546]
35. Collado M, Blasco MA, Serrano M. Cellular senescence in cancer and aging. Cell 2007;130:223. [PubMed: 17662938]
36. Collins CJ, Sedivy JM. Involvement of the INK4a/Arf gene locus in senescence. Aging Cell 2003;2:145. [PubMed: 12882406]
37. Das D, Scovell WM. The binding interaction of HMG-1 with the TATA-binding protein/TATA complex. J Biol Chem 2001;276:32597. [PubMed: 11390376]
38. Dasgupta A, Scovell WM. TFIIA abrogates the effects of inhibition by HMGB1 but not E1A during the early stages of assembly of the transcriptional preinitiation complex. Biochim Biophys Acta 2003;1627:101. [PubMed: 12818428]
39. De M, Visone IR, Fedele M, Petrocca F, Palmieri D, Martinez HJ, Forzati F, Croce CM, Fusco A. Regulation of microRNA expression by HMGA1 proteins. Oncogene 2009;28:1432. [PubMed: 19169275]
40. Dement GA, Treff NR, Magnuson NS, Franceschi V, Reeves R. Dynamic mitochondrial localization of nuclear transcription factor HMGA1. Exp Cell Res 2005;307:388. [PubMed: 15893306]
41. Diana F, Sgarra R, Manfioletti G, Rustighi A, Poletto D, Sciortino MT, Mastino A, Giancotti V. A link between apoptosis and degree of phosphorylation of high mobility group A1a protein in leukemic cells. J Biol Chem 2001;276:11354. [PubMed: 11145960]
42. Ding HF, Rimsky S, Batson SC, Bustin M, Hansen U. Stimulation of RNA polymerase II elongation by chromosomal protein HMG-14. Science 1994;265:796. [PubMed: 8047885]
43. Disney JE, Johnson KR, Magnuson NS, Sylvester SR, Reeves R. High-mobility group protein HMG-I localizes to G/Q- and C-bands of human and mouse chromosomes. J Cell Biol 1989;109:1975. [PubMed: 2808516]
44. Duncan B, Zhao K. HMGA1 mediates the activation of the CRYAB promoter by BRG1. DNA Cell Biol 2007;26:745. [PubMed: 17723105]
45. Dunker AK, Lawson JD, Brown CJ, Williams RM, Romero P, Oh JS, Oldfield CJ, Campen AM, Ratliff CM, Hipps KW, Ausio J, Nissen MS, Reeves R, Kang C, Kissinger CR, Bailey RW, Griswold MD, Chiu W, Garner EC, Obradovic Z. Intrinsically disordered protein. J Mol Graph Model 2001;19:26. [PubMed: 11381529]
46. Ellwood KB, Yen YM, Johnson RC, Carey M. Mechanism for specificity by HMG-1 in enhanceosome assembly. Mol Cell Biol 2000;20:4359. [PubMed: 10825199]
47. Falvo JV, Thanos D, Maniatis T. Reversal of intrinsic DNA bends in the IFN beta gene enhancer by transcription factors and the architectural protein HMG I(Y). Cell 1995;83:1101. [PubMed: 8548798]
48. Fashena SJ, Reeves R, Ruddle NH. A poly(dA-dT) upstream activating sequence binds high-mobility group I protein and contributes to lymphotoxin (tumor necrosis factor-beta) gene regulation. Mol Cell Biol 1992;12:894. [PubMed: 1732752]
49. Fedele M, Pentimalli F, Baldassarre G, Battista S, Klein-Szanto AJ, Kenyon L, Visone R, De M, Ciarmiello IA, Arra C, Viglietto G, Croce CM, Fusco A. Transgenic mice overexpressing the wild-type form of the HMGA1 gene develop mixed growth hormone/prolactin cell pituitary adenomas and natural killer cell lymphomas. Oncogene 2005;24:3427. [PubMed: 15735694]
50. Fedele M, Pierantoni GM, Berlingieri MT, Battista S, Baldassarre G, Munshi N, Dentice M, Thanos D, Santoro M, Viglietto G, Fusco A. Overexpression of proteins HMGA1 induces cell cycle deregulation and apoptosis in normal rat thyroid cells. Cancer Res 2001;61:4583. [PubMed: 11389094]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

51. Fedele M, Pierantoni GM, Visone R, Fusco A. Critical role of the HMGA2 gene in pituitary adenomas. Cell Cycle 2006;5:2045. [PubMed: 16969098]
52. Fedele M, Visone R, De M, Troncone IG, Palmieri D, Battista S, Ciarmiello A, Pallante P, Arra C, Melillo RM, Helin K, Croce CM, Fusco A. HMGA2 induces pituitary tumorigenesis by enhancing E2F1 activity. Cancer Cell 2006;9:459. [PubMed: 16766265]
53. Fousteri M, Vermeulen W, van Zeeland AA, Mullenders LH. Cockayne syndrome A and B proteins differentially regulate recruitment of chromatin remodeling and repair factors to stalled RNA polymerase II in vivo. Mol Cell 2006;23:471. [PubMed: 16916636]
54. Frasca F, Rustighi A, Malaguarnera R, Altamura S, Vigneri P, Del SG, Giancotti V, Pezzino V, Vigneri R, Manfioletti G. HMGA1 inhibits the function of p53 family members in thyroid cancer cells. Cancer Res 2006;66:2980. [PubMed: 16540646]
55. Friedmann M, Holth LT, Zoghbi HY, Reeves R. Organization, inducible- expression and chromosome localization of the human HMG-I(Y) nonhistone protein gene. Nucleic Acids Res 1993;21:4259. [PubMed: 8414980]
56. Funayama R, Saito M, Tanobe H, Ishikawa F. Loss of linker histone H1 in cellular senescence. J Cell Biol 2006;175:869. [PubMed: 17158953]
57. Furusawa T, Lim JH, Catez F, Birger Y, Mackem S, Bustin M. Down-regulation of nucleosomal binding protein HMGN1 expression during embryogenesis modulates Sox9 expression in chondrocytes. Mol Cell Biol 2006;26:592. [PubMed: 16382150]
58. Fusco A, Fedele M. Roles of HMGA proteins in cancer. Nat Rev Cancer 2007;7:899. [PubMed: 18004397]
59. Gabellini D, Green MR, Tupler R. Inappropriate gene activation in FSHD: a repressor complex binds a chromosomal repeat deleted in dystrophic muscle. Cell 2002;110:339. [PubMed: 12176321]
60. Gaffen SL. Signaling domains of the interleukin 2 receptor. Cytokine 2001;14:63. [PubMed: 11356007]
61. Gerlitz G, Hock R, Ueda T, Bustin M. The dynamics of HMG protein-chromatin interactions in living cells. Biochem Cell Biol 2009;87:127. [PubMed: 19234529]
62. Harrer M, Luhrs H, Bustin M, Scheer U, Hock R. Dynamic interaction of HMGA1a proteins with chromatin. J Cell Sci 2004;117:3459. [PubMed: 15213251]
63. He Q, Liang CH, Lippard SJ. Steroid hormones induce HMG1 overexpression and sensitize breast cancer cells to cisplatin and carboplatin. Proc Natl Acad Sci U S A 2000;97:5768. [PubMed: 10811891]
64. Henderson A, Bunce M, Siddon N, Reeves R, Tremethick DJ. High-mobility-group protein I can modulate binding of transcription factors to the U5 region of the human immunodeficiency virus type 1 proviral promoter. J Virol 2000;74:10523. [PubMed: 11044097]
65. Henderson A, Holloway A, Reeves R, Tremethick DJ. Recruitment of SWI/SNF to the human immunodeficiency virus type 1 promoter. Mol Cell Biol 2004;24:389. [PubMed: 14673171]
66. Hill DA, Pedulla ML, Reeves R. Directional binding of HMG-I(Y) on four-way junction DNA and the molecular basis for competitive binding with HMG-1 and histone H1. Nucleic Acids Res 1999;27:2135. [PubMed: 10219086]
67. Hill DA, Peterson CL, Imbalzano AN. Effects of HMGN1 on chromatin structure and SWI/SNF-mediated chromatin remodeling. J Biol Chem 2005;280:41777. [PubMed: 16253989]
68. Hill DA, Reeves R. Competition between HMG-I(Y), HMG-1 and histone H1 on four-way junction DNA. Nucleic Acids Res 1997;25:3523. [PubMed: 9254714]
69. Himes SR, Coles LS, Reeves R, Shannon MF. High mobility group protein I(Y) is required for function and for c-Rel binding to CD28 response elements within the GM-CSF and IL-2 promoters. Immunity 1996;5:479. [PubMed: 8934574]
70. Himes SR, Reeves R, Attema J, Nissen M, Li Y, Shannon MF. The role of high-mobility group I(Y) proteins in expression of IL-2 and T cell proliferation. J Immunol 2000;164:3157. [PubMed: 10706706]
71. Hock R, Furusawa T, Ueda T, Bustin M. HMG chromosomal proteins in development and disease. Trends Cell Biol 2007;17:72. [PubMed: 17169561]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

72. Hommura F, Katabami M, Leaner VD, Donninger H, Sumter TF, Resar LM, Birrer MJ. HMG-I/Y is a c-Jun/activator protein-1 target gene and is necessary for c-Jun-induced anchorage-independent growth in Rat1a cells. Mol Cancer Res 2004;2:305. [PubMed: 15192124]

73. Huth JR, Bewley CA, Nissen MS, Evans JN, Reeves R, Gronenborn AM, Clore GM. The solution structure of an HMG-I(Y)-DNA complex defines a new architectural minor groove binding motif. Nat Struct Biol 1997;4:657. [PubMed: 9253416]

74. Ito I, Fukazawa J, Yoshida M. Post-translational methylation of high mobility group box 1 (HMGB1) causes its cytoplasmic localization in neutrophils. J Biol Chem 2007;282:16336. [PubMed: 17403684]

75. Jeyapalan JC, Sedivy JM. Cellular senescence and organismal aging. Mech Ageing Dev 2008;129:467. [PubMed: 18502472]

76. John S, Reeves RB, Lin JX, Child R, Leiden JM, Thompson CB, Leonard WJ. Regulation of cell-type-specific interleukin-2 receptor alpha-chain gene expression: potential role of physical interactions between Elf-1, HMG-I(Y), and NF-kappa B family proteins. Mol Cell Biol 1995;15:1786. [PubMed: 7862168]

77. John S, Robbins CM, Leonard WJ. An IL-2 response element in the human IL-2 receptor alpha chain promoter is a composite element that binds Stat5, Elf-1, HMG-I(Y) and a GATA family protein. EMBO J 1996;15:5627. [PubMed: 8896456]

78. Kas E, Poljak L, Adachi Y, Laemmli UK. A model for chromatin opening: stimulation of topoisomerase II and restriction enzyme cleavage of chromatin by distamycin. EMBO J 1993;12:115. [PubMed: 8381347]

79. Knapp S, Muller S, Digilio G, Bonaldi T, Bianchi ME, Musco G. The long acidic tail of high mobility group box 1 (HMGB1) protein forms an extended and flexible structure that interacts with specific residues within and between the HMG boxes. Biochemistry 2004;43:11992. [PubMed: 15379539]

80. Korner U, Bustin M, Scheer U, Hock R. Developmental role of HMGN proteins in Xenopus laevis. Mech Dev 2003;120:1177. [PubMed: 14568106]

81. Krynetski EY, Krynetskaia NF, Bianchi ME, Evans WE. A nuclear protein complex containing high mobility group proteins B1 and B2, heat shock cognate protein 70, ERp60, and glyceraldehyde-3-phosphate dehydrogenase is involved in the cytotoxic response to DNA modified by incorporation of anticancer nucleoside analogues. Cancer Res 2003;63:100. [PubMed: 12517784]

82. Laemmli UK, Kas E, Poljak L, Adachi Y. Scaffold-associated regions: cis-acting determinants of chromatin structural loops and functional domains. Curr Opin Genet Dev 1992;2:275. [PubMed: 1322207]

83. Lafferty-Whyte K, Cairney CJ, Jamieson NB, Oien KA, Keith WN. Pathway analysis of senescence-associated miRNA targets reveals common processes to different senescence induction mechanisms. Biochim Biophys Acta 2009;1792:341. [PubMed: 19419692]

84. Lehtonen S, Lehtonen E. HMG-17 is an early marker of inductive interactions in the developing mouse kidney. Differentiation 2001;67:154. [PubMed: 11683498]

85. Liau SS, Ashley SW, Whang EE. Lentivirus-mediated RNA interference of HMGA1 promotes chemosensitivity to gemcitabine in pancreatic adenocarcinoma. J Gastrointest Surg 2006;10:1254. [PubMed: 17114012]

86. Liau SS, Jazag A, Ito K, Whang EE. Overexpression of HMGA1 promotes anoikis resistance and constitutive Akt activation in pancreatic adenocarcinoma cells. Br J Cancer 2007;96:993. [PubMed: 17342093]

87. Liau SS, Whang E. HMGA1 is a molecular determinant of chemoresistance to gemcitabine in pancreatic adenocarcinoma. Clin Cancer Res 2008;14:1470. [PubMed: 18316571]

88. Lim JH, Catez F, Birger Y, West KL, Prymakowska-Bosak M, Postnikov YV, Bustin M. Chromosomal protein HMGN1 modulates histone H3 phosphorylation. Mol Cell 2004;15:573. [PubMed: 15327773]

89. Lim JH, West KL, Rubinstein Y, Bergel M, Postnikov YV, Bustin M. Chromosomal protein HMGN1 enhances the acetylation of lysine 14 in histone H3. EMBO J 2005;24:3038. [PubMed: 16096646]

90. Liu R, Liu H, Chen X, Kirby M, Brown PO, Zhao K. Regulation of CSF1 promoter by the SWI/SNF-like BAF complex. Cell 2001;106:309. [PubMed: 11509180]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

91. Liu WM, Guerra-Vladusic FK, Kurakata S, Lupu R, Kohwi-Shigematsu T. HMG-I(Y) recognizes base-unpairing regions of matrix attachment sequences and its increased expression is directly linked to metastatic breast cancer phenotype. Cancer Res 1999;59:5695. [PubMed: 10582687]
92. Lomvardas S, Thanos D. Nucleosome sliding via TBP DNA binding in vivo. Cell 2001;106:685. [PubMed: 11572775]
93. Lomvardas S, Thanos D. Modifying gene expression programs by altering core promoter chromatin architecture. Cell 2002;110:261. [PubMed: 12150933]
94. Lucas J, Bilzer A, Moll L, Zundorf I, Dingermann T, Eichinger L, Siol O, Winckler T. The carboxy-terminal domain of Dictyostelium C-module-binding factor is an independent gene regulatory entity. PLoS One 2009;4:e5012. [PubMed: 19343174]
95. Lund T, Holtlund J, Fredriksen M, Laland SG. On the presence of two new high mobility group-like proteins in HeLa S3 cells. FEBS Lett 1983;152:163. [PubMed: 6297996]
96. Maffini M, Denes V, Sonnenschein C, Soto A, Geck P. APRIN is a unique Pds5 paralog with features of a chromatin regulator in hormonal differentiation. J Steroid Biochem Mol Biol 2008;108:32. [PubMed: 17997301]
97. Maloney SC, Adair JE, Smerdon MJ, Reeves R. Gene-specific nucleotide excision repair is impaired in human cells expressing elevated levels of high mobility group A1 nonhistone proteins. DNA Repair (Amst) 2007;6:1371. [PubMed: 17540622]
98. Mao L, Wertzler KJ, Maloney SC, Wang Z, Magnuson NS, Reeves R. HMGA1 Levels Influence Mitochondrial Function and mtDNA Repair Efficiency, Mol. Cell Biol. 2009 ePubl.
99. Merika M, Thanos D. Enhanceosomes. Curr Opin Genet Dev 2001;11:205. [PubMed: 11250145]
100. Merika M, Williams AJ, Chen G, Collins T, Thanos D. Recruitment of CBP/p300 by the IFN beta enhanceosome is required for synergistic activation of transcription. Mol Cell 1998;1:277. [PubMed: 9659924]
101. Mott JL, Kobayashi S, Bronk SF, Gores GJ. mir-29 regulates Mcl-1 protein expression and apoptosis. Oncogene 2007;26:6133. [PubMed: 17404574]
102. Munshi N, Agalioti T, Lomvardas S, Merika M, Chen G, Thanos D. Coordination of a transcriptional switch by HMGI(Y) acetylation. Science 2001;293:1133. [PubMed: 11498590]
103. Munshi N, Merika M, Yie J, Senger K, Chen G, Thanos D. Acetylation of HMG I(Y) by CBP turns off IFN beta expression by disrupting the enhanceosome. Mol Cell 1998;2:457. [PubMed: 9809067]
104. Munshi N, Yie Y, Merika M, Senger K, Lomvardas S, Agalioti T, Thanos D. The IFN-beta enhancer: a paradigm for understanding activation and repression of inducible gene expression. Cold Spring Harb Symp Quant Biol 1999;64:149. [PubMed: 11232280]
105. Najimi M, Smets F, Sokal E. Hepatocyte apoptosis. Methods Mol Biol 2009;481:59. [PubMed: 19096793]
106. Narita M. Cellular senescence and chromatin organisation. Br J Cancer 2007;96:686. [PubMed: 17311013]
107. Narita M, Narita M, Krizhanovsky V, Nunez S, Chicas A, Hearn SA, Myers MP, Lowe SW. A novel role for high-mobility group a proteins in cellular senescence and heterochromatin formation. Cell 2006;126:503. [PubMed: 16901784]
108. Narita M, Nunez S, Heard E, Narita M, Lin AW, Hearn SA, Spector DL, Hannon GJ, Lowe SW. Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. Cell 2003;113:703. [PubMed: 12809602]
109. Nissen MS, Langan TA, Reeves R. Phosphorylation by cdc2 kinase modulates DNA binding activity of high mobility group I nonhistone chromatin protein. J Biol Chem 1991;266:19945. [PubMed: 1939057]
110. Nissen MS, Reeves R. Changes in superhelicity are introduced into closed circular DNA by binding of high mobility group protein I/Y. J Biol Chem 1995;270:4355. [PubMed: 7876198]
111. Ogram SA, Reeves R. Differential regulation of a multipromoter gene. Selective 12-O-tetradecanoylphorbol-13-acetate induction of a single transcription start site in the HMG-I/Y gene. J Biol Chem 1995;270:14235. [PubMed: 7775485]
112. Ohtani N, Mann DJ, Hara E. Cellular senescence: its role in tumor suppression and aging. Cancer Sci 2009;100:792. [PubMed: 19302284]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

113. Ohtani N, Yamakoshi K, Takahashi A, Hara E. The p16INK4a-RB pathway: molecular link between cellular senescence and tumor suppression. J Med Invest 2004;51:146. [PubMed: 15460900]
114. Palvimo J, Maenpaa PH. Binding of high-mobility-group proteins HMG 14 and HMG 17 to DNA and histone H1 as influenced by phosphorylation. Biochim Biophys Acta 1988;952:172. [PubMed: 3337824]
115. Panne D. The enhanceosome. Curr Opin Struct Biol 2008;18:236. [PubMed: 18206362]
116. Paranjape SM, Krumm A, Kadonaga JT. HMG17 is a chromatin-specific transcriptional coactivator that increases the efficiency of transcription initiation. Genes Dev 1995;9:1978. [PubMed: 7649479]
117. Pash JM, Alfonso PJ, Bustin M. Aberrant expression of high mobility group chromosomal protein 14 affects cellular differentiation. J Biol Chem 1993;268:13632. [PubMed: 8514795]
118. Passos JF, Simillion C, Hallinan J, Wipat A, von ZT. Cellular senescence: unravelling complexity, Age (Dordr). 2009
119. Phair RD, Scaffidi P, Elbi C, Vecerova J, Dey A, Ozato K, Brown DT, Hager G, Bustin M, Misteli T. Global nature of dynamic protein-chromatin interactions in vivo: three-dimensional genome scanning and dynamic interaction networks of chromatin proteins. Mol Cell Biol 2004;24:6393. [PubMed: 15226439]
120. Pierantoni GM, Rinaldo C, Esposito F, Mottolese M, Soddu S, Fusco A. High Mobility Group A1 (HMGA1) proteins interact with p53 and inhibit its apoptotic activity. Cell Death Differ 2006;13:1554. [PubMed: 16341121]
121. Pierantoni GM, Rinaldo C, Mottolese M, Di BA, Esposito F, Soddu S, Fusco A. High-mobility group A1 inhibits p53 by cytoplasmic relocalization of its proapoptotic activator HIPK2. J Clin Invest 2007;117:693. [PubMed: 17290307]
122. Pil PM, Lippard SJ. Specific binding of chromosomal protein HMG1 to DNA damaged by the anticancer drug cisplatin. Science 1992;256:234. [PubMed: 1566071]
123. Postnikov YV, Belova GI, Lim JH, Bustin M. Chromosomal protein HMGN1 modulates the phosphorylation of serine 1 in histone H2A. Biochemistry 2006;45:15092. [PubMed: 17154547]
124. Postnikov YV, Herrera JE, Hock R, Scheer U, Bustin M. Clusters of nucleosomes containing chromosomal protein HMG-17 in chromatin. J Mol Biol 1997;274:454. [PubMed: 9417927]
125. Postnikov YV, Trieschmann L, Rickers A, Bustin M. Homodimers of chromosomal proteins HMG-14 and HMG-17 in nucleosome cores. J Mol Biol 1995;252:423. [PubMed: 7563062]
126. Pruss D, Reeves R, Bushman FD, Wolffe AP. The influence of DNA and nucleosome structure on integration events directed by HIV integrase. J Biol Chem 1994;269:25031. [PubMed: 7929189]
127. Prymakowska-Bosak M, Hock R, Catez F, Lim JH, Birger Y, Shirakawa H, Lee K, Bustin M. Mitotic phosphorylation of chromosomal protein HMGN1 inhibits nuclear import and promotes interaction with 14.3.3 proteins. Mol Cell Biol 2002;22:6809. [PubMed: 12215538]
128. Prymakowska-Bosak M, Misteli T, Herrera JE, Shirakawa H, Birger Y, Garfield S, Bustin M. Mitotic phosphorylation prevents the binding of HMGN proteins to chromatin. Mol Cell Biol 2001;21:5169. [PubMed: 11438671]
129. Radic MZ, Saghbini M, Elton TS, Reeves R, Hamkalo BA. Hoechst 33258, distamycin A, and high mobility group protein I (HMG-I) compete for binding to mouse satellite DNA. Chromosoma 1992;101:602. [PubMed: 1385053]
130. Rattner BP, Yusufzai T, Kadonaga JT. HMGN proteins act in opposition to ATP-dependent chromatin remodeling factors to restrict nucleosome mobility. Mol Cell 2009;34:620. [PubMed: 19524541]
131. Reeves R. Structure and function of the HMGI(Y) family of architectural transcription factors. Environ Health Perspect 2000;108(Suppl 5):803. [PubMed: 11035986]
132. Reeves R. Molecular biology of HMGA proteins: hubs of nuclear function. Gene 2001;277:63. [PubMed: 11602345]
133. Reeves R. HMGA proteins: flexibility finds a nuclear niche? Biochem Cell Biol 2003;81:185. [PubMed: 12897853]
134. Reeves R, Adair JE. Role of high mobility group (HMG) chromatin proteins in DNA repair. DNA Repair (Amst) 2005;4:926. [PubMed: 15916927]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

135. Reeves R, Beckerbauer L. HMGI/Y proteins: flexible regulators of transcription and chromatin structure. Biochim Biophys Acta 2001;1519:13. [PubMed: 11406267]
136. Reeves R, Langan TA, Nissen MS. Phosphorylation of the DNA-binding domain of nonhistone high-mobility group I protein by cdc2 kinase: reduction of binding affinity. Proc Natl Acad Sci U S A 1991;88:1671. [PubMed: 2000376]
137. Reeves R, Leonard WJ, Nissen MS. Binding of HMG-I(Y) imparts architectural specificity to a positioned nucleosome on the promoter of the human interleukin-2 receptor alpha gene. Mol Cell Biol 2000;20:4666. [PubMed: 10848593]
138. Reeves R, Nissen MS. The A.T-DNA-binding domain of mammalian high mobility group I chromosomal proteins. A novel peptide motif for recognizing DNA structure. J Biol Chem 1990;265:8573. [PubMed: 1692833]
139. Reeves R, Nissen MS. Interaction of high mobility group-I (Y) nonhistone proteins with nucleosome core particles. J Biol Chem 1993;268:21137. [PubMed: 8407950]
140. Reeves R, Nissen MS. Cell cycle regulation and functions of HMG-I(Y). Prog Cell Cycle Res 1995;1:339. [PubMed: 9552376]
141. Reeves R, Wolffe AP. Substrate structure influences binding of the non-histone protein HMG-I(Y) to free nucleosomal DNA. Biochemistry 1996;35:5063. [PubMed: 8664299]
142. Richards HW, Medrano EE. Epigenetic marks in melanoma. Pigment Cell Melanoma Res 2009;22:14. [PubMed: 19040501]
143. Saitoh Y, Laemmli UK. Metaphase chromosome structure: bands arise from a differential folding path of the highly AT-rich scaffold. Cell 1994;76:609. [PubMed: 7510215]
144. Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature 2002;418:191. [PubMed: 12110890]
145. Sedivy JM, Banumathy G, Adams PD. Aging by epigenetics--a consequence of chromatin damage? Exp Cell Res 2008;314:1909. [PubMed: 18423606]
146. Sgarra R, Diana F, Bellarosa C, Dekleva V, Rustighi A, Toller M, Manfioletti G, Giancotti V. During apoptosis of tumor cells HMGA1a protein undergoes methylation: identification of the modification site by mass spectrometry. Biochemistry 2003;42:3575. [PubMed: 12653562]
147. Sgarra R, Diana F, Rustighi A, Manfioletti G, Giancotti V. Increase of HMGA1a protein methylation is a distinctive characteristic of leukaemic cells induced to undergo apoptosis. Cell Death Differ 2003;10:386. [PubMed: 12700639]
148. Sgarra R, Lee J, Tessari MA, Altamura S, Spolaore B, Giancotti V, Bedford MT, Manfioletti G. The AT-hook of the chromatin architectural transcription factor high mobility group A1a is arginine-methylated by protein arginine methyltransferase 6. J Biol Chem 2006;281:3764. [PubMed: 16293633]
149. Sgarra R, Rustighi A, Tessari MA, Di Bernardo J, Altamura S, Fusco A, Manfioletti G, Giancotti V. Nuclear phosphoproteins HMGA and their relationship with chromatin structure and cancer. FEBS Lett 2004;574:1. [PubMed: 15358530]
150. Singh M, D'Silva L, Holak TA. DNA-binding properties of the recombinant high-mobility-group-like AT-hook-containing region from human BRG1 protein. Biol Chem 2006;387:1469. [PubMed: 17081121]
151. Smith KA. Interleukin-2. Curr Opin Immunol 1992;4:271. [PubMed: 1418705]
152. Soloaga A, Thomson S, Wiggin GR, Rampersaud N, Dyson MH, Hazzalin CA, Mahadevan LC, Arthur JS. MSK2 and MSK1 mediate the mitogen- and stress-induced phosphorylation of histone H3 and HMG-14. EMBO J 2003;22:2788. [PubMed: 12773393]
153. Solomon MJ, Strauss F, Varshavsky A. A mammalian high mobility group protein recognizes any stretch of six A.T base pairs in duplex DNA. Proc Natl Acad Sci U S A 1986;83:1276. [PubMed: 3456586]
154. Strick R, Laemmli UK. SARs are cis DNA elements of chromosome dynamics: synthesis of a SAR repressor protein. Cell 1995;83:1137. [PubMed: 8548801]
155. Su Y, Kwon CS, Bezhani S, Huvermann B, Chen C, Peragine A, Kennedy JF, Wagner D. The N-terminal ATPase AT-hook-containing region of the Arabidopsis chromatin-remodeling protein SPLAYED is sufficient for biological activity. Plant J 2006;46:685. [PubMed: 16640604]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

156. Tessari MA, Gostissa M, Altamura S, Sgarra R, Rustighi A, Salvagno C, Caretti G, Imbriano C, Mantovani R, Del SG, Giancotti V, Manfioletti G. Transcriptional activation of the cyclin A gene by the architectural transcription factor HMGA2. Mol Cell Biol 2003;23:9104. [PubMed: 14645522]

157. Thanos D, Maniatis T. Virus induction of human IFN beta gene expression requires the assembly of an enhanceosome. Cell 1995;83:1091. [PubMed: 8548797]

158. Thomas JO, Travers AA. HMG1 and 2, and related 'architectural' DNA-binding proteins. Trends Biochem Sci 2001;26:167. [PubMed: 11246022]

159. Travers AA. Priming the nucleosome: a role for HMGB proteins? EMBO Rep 2003;4:131. [PubMed: 12612600]

160. Trieschmann L, Alfonso PJ, Crippa MP, Wolffe AP, Bustin M. Incorporation of chromosomal proteins HMG-14/HMG-17 into nascent nucleosomes induces an extended chromatin conformation and enhances the utilization of active transcription complexes. EMBO J 1995;14:1478. [PubMed: 7729423]

161. Ueda T, Postnikov YV, Bustin M. Distinct domains in high mobility group N variants modulate specific chromatin modifications. J Biol Chem 2006;281:10182. [PubMed: 16484217]

162. Vallone D, Battista S, Pierantoni GM, Fedele M, Casalino L, Santoro M, Viglietto G, Fusco A, Verde P. Neoplastic transformation of rat thyroid cells requires the junB and fra-1 gene induction which is dependent on the HMGI-C gene product. EMBO J 1997;16:5310. [PubMed: 9311991]

163. Wang H, Bloom O, Zhang M, Vishnubhakat JM, Ombrellino M, Che J, Frazier A, Yang H, Ivanova S, Borovikova L, Manogue KR, Faist E, Abraham E, Andersson J, Andersson U, Molina PE, Abumrad NN, Sama A, Tracey KJ. HMG-1 as a late mediator of endotoxin lethality in mice. Science 1999;285:248. [PubMed: 10398600]

164. Wegner M, Grummt F. Netropsin, distamycin and berenil interact differentially with a high-affinity binding site for the high mobility group protein HMG-I. Biochem Biophys Res Commun 1990;166:1110. [PubMed: 2154972]

165. West KL. HMGN proteins play roles in DNA repair and gene expression in mammalian cells. Biochem Soc Trans 2004;32:918. [PubMed: 15506924]

166. West KL, Castellini MA, Duncan MK, Bustin M. Chromosomal proteins HMGN3a and HMGN3b regulate the expression of glycine transporter 1. Mol Cell Biol 2004;24:3747. [PubMed: 15082770]

167. Williams RS, Williams JS, Tainer JA. Mre11-Rad50-Nbs1 is a keystone complex connecting DNA repair machinery, double-strand break signaling, and the chromatin template. Biochem Cell Biol 2007;85:509. [PubMed: 17713585]

168. Wood LJ, Maher JF, Bunton TE, Resar LM. The oncogenic properties of the HMG-I gene family. Cancer Res 2000;60:4256. [PubMed: 10945639]

169. Wood LJ, Mukherjee M, Dolde CE, Xu Y, Maher JF, Bunton TE, Williams JB, Resar LM. HMG-I/Y, a new c-Myc target gene and potential oncogene. Mol Cell Biol 2000;20:5490. [PubMed: 10891489]

170. Xiao H, Sandaltzopoulos R, Wang HM, Hamiche A, Ranallo R, Lee KM, Fu D, Wu C. Dual functions of largest NURF subunit NURF301 in nucleosome sliding and transcription factor interactions. Mol Cell 2001;8:531. [PubMed: 11583616]

171. Xu Y, Sumter TF, Bhattacharya R, Tesfaye A, Fuchs EJ, Wood LJ, Huso DL, Resar LM. The HMG-I oncogene causes highly penetrant, aggressive lymphoid malignancy in transgenic mice and is overexpressed in human leukemia. Cancer Res 2004;64:3371. [PubMed: 15150086]

172. Yie J, Merika M, Munshi N, Chen G, Thanos D. The role of HMG I(Y) in the assembly and function of the IFN-beta enhanceosome. EMBO J 1999;18:3074. [PubMed: 10357819]

173. Yie J, Senger K, Thanos D. Mechanism by which the IFN-beta enhanceosome activates transcription. Proc Natl Acad Sci U S A 1999;96:13108. [PubMed: 10557281]

174. Youn JH, Shin JS. Nucleocytoplasmic shuttling of HMGB1 is regulated by phosphorylation that redirects it toward secretion. J Immunol 2006;177:7889. [PubMed: 17114460]

175. Zhang Q, Wang Y. High mobility group proteins and their post-translational modifications. Biochim Biophys Acta 2008;1784:1159. [PubMed: 18513496]

176. Zhang R, Chen W, Adams PD. Molecular dissection of formation of senescence-associated heterochromatin foci. Mol Cell Biol 2007;27:2343. [PubMed: 17242207]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

177. Zhang R, Poustovoitov MV, Ye X, Santos HA, Chen W, Daganzo SM, Erzberger JP, Serebriiskii IG, Canutescu AA, Dunbrack RL, Pehrson JR, Berger JM, Kaufman PD, Adams PD. Formation of MacroH2A-containing senescence-associated heterochromatin foci and senescence driven by ASF1a and HIRA. Dev Cell 2005;8:19. [PubMed: 15621527]
178. Zhao K, Kas E, Gonzalez E, Laemmli UK. SAR-dependent mobilization of histone H1 by HMG-I/Y in vitro: HMG-I/Y is enriched in H1-depleted chromatin. EMBO J 1993;12:3237. [PubMed: 8344261]
179. Zhou X, Benson KF, Ashar HR, Chada K. Mutation responsible for the mouse pygmy phenotype in the developmentally regulated factor HMGI-C. Nature 1995;376:771. [PubMed: 7651535]
180. Zlatanova J, van HK. Linker histones versus HMG1/2: a struggle for dominance? Bioessays 1998;20:584. [PubMed: 9723008]