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HMGN5/NSBP1: A New Member of the HMGN Protein Family That Affects Chromatin Structure and Function

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Abstract

The dynamic nature of the chromatin fiber provides the structural and functional flexibility required for the accurate transcriptional responses to various stimuli. In living cells, structural proteins such as the linker histone H1 and the High Mobility Group (HMG) proteins continuously modulate the local and global architecture of the chromatin fiber and affect the binding of regulatory factors to their nucleosomal targets. HMGN proteins specifically bind to the nucleosome core particle through a highly conserved "nucleosomal binding domain" (NBD) and reduce chromatin compaction. HMGN5 (NSBP1), a new member of the HMGN protein family, is ubiquitously expressed in mouse and human tissues. Similar to other HMGNs, HMGN5 is a nuclear protein which binds to nucleosomes via NBD, unfolds chromatin and affects transcription. This protein remains mainly uncharacterized and its biological function is unknown. In this review we describe the structure of the *HMGN5* gene and the known properties of the HMGN5 protein. We present recent findings related to the expression pattern of the protein during development, the mechanism of HMGN5 action on chromatin, and discuss the possible role of HMGN5 in pathological and physiological processes.

Keywords

HMGN5; NSBP1; chromatin; linker histone H1; transcription; nucleosome

1. Introduction

Essential cellular processes such as transcription, DNA repair, replication and recombination occur in the context of chromatin. Therefore, the architecture of the chromatin fiber plays a key role in the integration of endogenous and exogenous signals for proper adjustment of cellular responses to a constantly changing environment.

A hallmark of chromatin organization is the dynamic nature of the chromatin fiber [1,2]. Histones in the nucleosome core particle, the basic unit of chromatin, are subjected to numerous post translational modifications, collectively known as the "histone code" that directly affects the structure of chromatin [3]. The pattern of histone modifications is established and maintained through the balancing action of chromatin modifying enzymes that continuously add and remove modifications to histone tails. Histone modifications are recognized by

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chromatin remodeling complexes thereby providing the molecular platform for chromatin remodeling [4]. The next level of complexity of chromatin fiber is the formation of higher order chromatin structures, such as the 30 nm fiber, tertiary structures of chromatin loops, and entire chromosomes [5]. The dynamic properties of these structures are regulated by numerous chromatin modifying factors including a complex network of nucleosome-binding chromatin architectural proteins, such as linker histone H1 [6] and High Mobility Group (HMG) proteins [7]. The activity of the linker histone H1 is required for the formation of higher order chromatin structures and chromatin compaction whereas HMGNs, such as HMGN1 and HMGN2 compete with histone H1 for the binding to nucleosomes and counteract this function [7–9, 14].

High Mobility Group N (HMGN) proteins are a family of ubiquitous nuclear proteins which are expressed in higher eukaryotes and specifically interact with nucleosomes without any known preference for the underlying DNA [10]. This interaction is mediated by the Nucleosome Binding Domain (NBD), a highly conserved sequence motif that is the signature of the HMGN protein family. Additionally, all members of the HMGN family contain negatively charged and highly unstructured C-terminal regulatory domain which increases the affinity of HMGN binding to the nucleosome and is involved in transcriptional activation [11]. However, the molecular function of this domain is not fully understood. Based on these structural properties, six proteins have been identified as members of HMGN protein family: HMGN1 (HMG14), HMGN2 (HMG17), HMGN3a and 3b, HMGN4, and HMGN5, previously known as NBP-45, GARP45 and NSBP1.

Fluorescence Recovery after Photobleaching (FRAP) experiments demonstrate that HMGN proteins are highly dynamic and are constantly moving in the nucleus in a “stop and go” manner [12]. HMGNs destabilize linker histone H1 binding to nucleosomes by competing for chromatin binding sites [9], unfold higher order chromatin structures [13,14] and modulate transcription [15,16]. In addition, HMGNs affect several histone modifications thus adjusting the chromatin architecture at the level of the nucleosome [17–19]. Also, it has been recently reported that recombinant HMGN proteins can counteract the ATP-dependent chromatin remodeling activities in vitro in reversible and dynamic manner [20].

Studies of HMGN1 knock out (KO) mice reveal that these mice are hypersensitive to UV and ionizing radiation and have a higher incidence of multiple malignant tumors and metastases [21,22] The underlying molecular mechanism for the increased tumor burden of HMGN1 KO mice lies in the ability of HMGN1 to optimize the interaction of the DNA repair machinery with chromatin [23]. Aberrant expression of HMGN proteins during mouse development and cellular differentiation is detrimental for early embryonic development, myotube formation and chondrocyte differentiation, indicating that proper regulation of HMGN protein expression is important for these processes [16,24,25].

HMGN5 protein was first identified in our laboratory based on its structural similarity to other HMGN proteins [26]. HMGN5 is a typical member of the HMGN family which is localized to the nucleus and contains a functional NBD and negatively charged C-terminus [26]. Unlike other HMGNs, the C-terminus of HMGN5 is unusually long (more than 300 amino acids long in the mouse protein and around 200 amino acids long in the human protein) and affects cellular localization and architectural properties of the protein [27].

HMGN5 remains mainly uncharacterized and its biological function is unknown. In this review we summarize the available data on molecular and biochemical properties of HMGN5 gene and protein, expression pattern during development and its role in the regulation of chromatin architecture and transcription. We discuss the potential association of HMGN5 with physiological and pathological processes, including cancer progression. We note that most of

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the recent data come from work on the mouse HMGN5 protein and further research is required to elucidate the functional importance of this unusual chromatin architectural protein in other species.

2. The structure of the HMGN5 gene

Analysis of the databases of ortholog genes suggest that the *Hmgn5* gene appeared late in evolution and is only present in rats, mice, cows, monkeys and human. For other species such as dogs, pigs, chicken and lower eukaryotes no similar gene or protein are predicted. Genes coding for both mouse and human HMGN5 proteins are located at syntenic regions of the X chromosome (Xq13.3 for human gene and X D for mouse gene, Fig 1A) and span approximately 8 Kb regions. In contrast to the *Hmgn1*, which has multiple copies of non functional retropseudogenes *Hmgn5* gene is present in the genome in a single copy [28]. The open reading frame of *Hmgn5* is flanked by a short 5′ UTR and a long 3′ UTR which contains several polyadenylation signals. *Hmgn5* is composed of 6 exons and 5 introns which encode a 282 amino acids long human protein and 406 amino acids long mouse protein. A significantly shorter exon 6 of the human *HMGN5* gene accounts for the length difference between the two proteins (Fig 1B). In humans the protein region encoded by the last exon of *HMGN5* contains sequences highly similar to HAL1 and LINE1 retrotransposable elements and sequences corresponding to the HERVH endogenous retrovirus [28].

3. The properties of the HMGN5 protein

Human and mouse HMGN5 proteins show 59% amino acid identity (86% similarity) and are structurally similar (Fig 1C). The N-terminal part of HMGN5 contains a nuclear localization signal and the NBD. RRSARLSA is the highly conserved functional domain of the NBD which defines the ability of HMGN proteins to specifically interact with nucleosomes [29]. Additional features of HMGN5 associated with HMGN origin include the asymmetric charge distribution along the molecule: the N-terminal region containing NBD is positively charged, whereas C-terminal tail is highly acidic [10]. Analysis of the sequence of HMGN5 protein also predicts the existence of several coiled coil structures which may facilitate interaction with yet unidentified protein partners (Fig. 1C).

Specific binding of HMGN proteins to nucleosomes has been detected by mobility shift assay with purified proteins and core particles [30]. At low ionic strength (0.5X TBE) HMGN1 binds to core particles in noncooperative manner and forms two distinct complexes corresponding to the binding of one or two molecules of HMGN1 per core particle. At “physiological” ionic strength (2X TBE) the binding of HMGN1 is cooperative and only complexes with two HMGN1 molecules per core particle are observed [30]. As expected, under “physiological” conditions HMGN5 cooperatively binds to core particles and forms a complex of 2 molecules of HMGN5 per one core particle with a dissociation constant similar to the HMGN1 ($0.4\times10^{-7} \times m^{-1}$) [26]. Interestingly, in contrast to HMGN1, at low ionic strength HMGN5 does not reveal a noncooperative binding [26]. Importantly, mutations in two serine residues in the core sequence of the NBD of HMGN5, as well as other HMGN proteins, abrogate the interaction of HMGNs with the nucleosome core particles [26,29,31].

It has been demonstrated in cells that different members of HMGN protein family cluster into distinct chromatin domains [32]. Nucleosomes containing a heterodimer, i.e. two different HMGN proteins on the single core particle have not been detected either in vitro or in vivo [33]. In agreement with this observation, mobility shift assays indicate that HMGN1 and HMGN5 form separate complexes with core particles [26].

The unique structural feature of HMGN5 is the long and highly acidic C-terminus which contains a stretch of amino acid repeats with a strong consensus sequence EEDGKE for the

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mouse protein and a more degenerative consensus for the human protein (Fig 1C). These repeats are present 13 times in mouse and 11 times in human HMGN5. Though the biological role of these repeats is not known, the long acidic tail of mouse HMGN5 was associated with general transcriptional activation activity and increased HMGN5-nucleosome interaction in the mobility shift assay [26].

As predicted from its protein sequence, HMGN5, especially its negatively charged C-terminal domain, is highly disordered in solution suggesting that the protein does not have a well defined conformation under native conditions [27]. Long, intrinsically disordered regions are found in around 30% of eukaryotic proteins [34]. For example, linker histone H1, histone acetyltransferase p300 and c-AMP response element binding protein (CREB) contain highly disordered sequences which are required for the proper function of these proteins [35,36]. This structural feature of HMGN5 is important for understanding its genome wide effect on gene expression. Disordered proteins are prone to form promiscuous low affinity complexes with multiple targets [37]. These proteins are especially common in the regulation of transcription, cell cycle and chaperone folding of proteins. Recently it was demonstrated that the increased sensitivity of cells to overexpression of certain genes is highly correlated with the intrinsically disordered structure of the expressed proteins [37]. When overexpressed, such intrinsically disordered proteins were predicted to have a significant phenotypic effect due to promiscuous molecular interactions. In agreement with this interaction promiscuity theory, overexpression of highly disordered HMGN1 protein inhibited myotube formation and chondrocyte differentiation in cultured cells [16,25], whereas HMGN1 knock out cells behave normally unless subjected to stress [21]. We also observed that elevated expression of HMGN5 leads to formation of hyperfused myotubes following differentiation of C2C12 myoblasts in vitro (Rochman, M., unpublished observations) and significantly changes cellular transcription profile [27].

4. Intracellular localization of HMGN5

As other HMGN proteins, HMGN5 is also localized to the nucleus of the cell [26]. More detailed analysis reveals that mouse HMGN5 protein is specifically localized to less condensed euchromatic areas and is excluded from more condensed constitutive heterochromatin domains [27]. This localization is unusual because another member of the HMGN family, HMGN1, localizes to both eu- and heterochromatic areas of the nucleus [29]. The major structural distinction between these proteins which accounts for the different nuclear localization is the long acidic C-terminal tail of HMGN5. Indeed, by gradual deletions of the C-terminal tail of HMGN5 it was shown that the tail serves as a module that targets HMGN5 to euchromatin [27]. However, this function of the tail is limited to HMGN proteins. The heterochromatic localization of Heterochromatic Protein 1 (HP1) was not affected by the addition of HMGN5 tail, whereas HMGN1 was relocalized to euchromatin [27]. Moreover, the targeting effect of the acidic tail of HMGN5 protein cannot be substituted by the acidic tail of Acidic Repeat Containing (ACRC) protein, suggesting the importance of the sequence of the HMGN5 tail rather than its charge. Interestingly, intranuclear localization of the human HMGN5 protein, which has a significantly shorter acidic tail, is not restricted to euchromatin (Malicet, C., unpublished observations).

5. Expression pattern of HMGN5 during development

Despite the ubiquitous expression of HMGN5, expression levels of the protein vary significantly between tissues and cell lines [26,28]. A query of the Gene Expression Omnibus (GEO) expression database suggests the highest expression of mouse HMGN5 protein in the pituitary gland, which is supported by the relatively high expression of the protein in the AtT20 mouse pituitary cell line [27].

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As indicated by mRNA and protein analysis, both human and mouse HMGN5 proteins are ubiquitously expressed in adult tissues, although the expression varies during embryonic development [38]. At early stages of mouse development, up to blastocyst stage, HMGN5 is ubiquitously expressed; however starting from 7.5 days post coitum (dpc) its expression is detectable predominantly in ectoplacental cone, an extra embryonic precursor of the placenta [38]. In contrast, HMGN1 protein was mainly localized to the embryonic tissues and excluded from the ectoplacental cone. At 9.5–11.5 dpc HMGN5 transcripts are detected in the giant trophoblast, spongiotrophoblast and decidual cells of the placenta, while expression in the developing embryo remains relatively weak [38]. The most prominent expression was detected in trophoblast giant cells. This expression pattern is recapitulated in rat choriocarcinoma Rcho1 cells, an in vitro model of trophoblast giant cell differentiation. Using this model, it was demonstrated that deregulation of HMGN5 expression by overexpression or siRNA-mediated down regulation altered the expression of prominent markers of giant cell differentiation, suggesting the role of HMGN5 in placental differentiation and function [38]. Consistent with this idea, expression array analysis revealed the two fold increase in HMGN5 transcript in the implantation sites of mice [39]. We also observed similar increase of HMGN5 by immunofluorescence studies (Furusawa, T., unpublished observations).

6. HMGN5 unfolds chromatin and modulates transcription

The concept of dynamic chromatin interactions is paramount in understanding how a relatively low amount of protein (sufficient to bind to less than 2% of the nucleosomes) has a global effect on chromatin architecture [40]. As evident from FRAP analysis, HMGN proteins are highly mobile. They are constantly moving through the nucleus, randomly interacting with nucleosomes and forming metastable protein complexes [8,41]. The mobility properties of HMGNs are defined by the binding of the proteins to nucleosomes, as apparent from the increased mobility rate of the proteins which are mutated in the NBD and do not bind to nucleosomes [29]. Following binding to chromatin, HMGNs reduce the compaction of higher order chromatin structures thereby affecting transcription and DNA repair [13,23].

From this perspective, HMGN5 is a characteristic member of the HMGN protein family. It moves very fast through the nucleus and may be the fastest moving member of the HMGN protein family described so far [27]. Altered expression levels of mouse HMGN5 affect transcription of specific markers of differentiation in Rcho-1 placental cells [38] and have a global effect on transcription of pituitary AtT20 cells [27]. Importantly, overexpression of HMGN5 at only twice the endogenous protein level in AtT20 cells alters the expression of more than 2,500 genes. This effect correlates with the ability of HMGN5 to unfold chromatin in vivo and counteract linker histone-mediated chromatin compaction in vitro. Transcription changes induced by HMGN5 are linked to the ability of the protein to interact with nucleosomes because a mutated HMGN5 which does not bind to the nucleosome has no effect on transcription [27]. Though HMGN5 affects the expression of many genes, the magnitude of the effect on each gene is moderate. This suggests that HMGN5 modulates transcription fidelity of the cell.

Alterations in chromatin architecture induced by the binding of HMGN5 provide a molecular explanation for the effects of the protein on transcription and other cellular processes. The effect of HMGN5 binding on chromatin architecture was assessed in a well characterized LacO-LacR array system [42] (Fig 2A). In this system, 256 copies of the LacO sequence are stably integrated into the genome of cells. Fusion of the protein of interest to LacR will tether the protein to LacO sequence and can induce alterations in local chromatin architecture of the array which are detected by fluorescence microscopy. Tethering of wild type HMGN5 protein to the LacO array caused dramatic decondensation of an otherwise compact array structure (Fig 2A), which resembles the unlooping of higher order chromatin structures by the viral

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transcriptional activator VP16 [43]. The decondensation effect of HMGN5 on the chromatin fiber was supported by increased sensitivity of the chromatin to micrococcal nuclease digestion and by visualization of global chromatin reorganization by microscopy. Significantly, the reorganization and unfolding of chromatin fiber required the proper interaction of HMGN5 with the nucleosome [27].

The ability of HMGN5 to unfold chromatin, its overlapping binding site on the nucleosome, and its unusually long acidic tail suggest that direct interaction of HMGN5 with linker histone H1 may be a part of the molecular mechanism of HMGN5-mediated chromatin unfolding. Indeed, a direct interaction between negatively charged HMGN5 tail and positively charged C-terminus of histone H5 (a variant of linker histone H1) was demonstrated both in vitro and in vivo [27]. Significantly, this interaction was not observed for HMGN1, the protein which lacks a long, negatively charged C-terminus uniquely present in HMGN5. In agreement with this data, HMGN5 but not HMGN1 could effectively counteract the compacting effect of linker H5 on a chromatin array in vitro. Similar to HMGN5, it has been recently reported that HMGB1 protein and histone H1 interact in vitro through their acidic and basic tails and that this interaction has the potential to affect global chromatin architecture in the cell [44]. Same kind of acidic-basic interaction was also shown for prothymosin alpha [45], a highly conserved acidic polypeptide localized to the cell nucleus [46].

A major remaining question however is how HMGN5 affects chromatin architecture and transcription if the protein can only bind to a small fraction of the nucleosomes. Our model (Fig 2B) predicts that in the nucleus HMGN5 and linker histones are constantly moving and randomly collide on the nucleosomes. This brief encounter leads to two molecular events: first is a competition of the NBD of HMGN5 with the globular domain of linker histone H1 for the binding site on the nucleosome and a second is a transient interaction of these proteins through their tails (Fig 2C). Subsequently, the efficiency of binding of linker histone H1 to the nucleosome is decreased and chromatin is transiently decondensed. This decondensation can be limited to certain nucleosomes or can have a more global effect if the formation of higher order chromatin structures such as chromatin loops is affected. We believe that HMGN5 can serve as part of multiprotein complexes which determine the preferential association of HMGN5 with defined chromatin regions. Indeed, preferential localization of HMGN1 to the promoters of active genes was suggested [47].

The ability of HMGNs to compete with the binding of linker histones to chromatin is contingent on the correct binding of HMGNs to nucleosomes, because HMGN1 mutants that do not bind to nucleosomes, do not compete with linker histone H1 [8]. However, the contribution of individual structural domains of HMGNs, such as NBD or regulatory domain to this competition is not clear. It has been shown that a C-terminal deletion mutant of HMGN1 is ineffective in competing with linker histone H1 in mixed chromatin domains [8]. On the other hand, the mobility of tailless HMGN5 mutant is significantly faster that that of HMGN1 [27], implying that the sequence differences in the regions adjacent to the core NDB contribute to different functional properties of HMGNs. Thus, HMGNs differ in their localization pattern, nuclear mobility [27], effect on chromatin modifications [48] and chromatin compaction [27]. Furthermore, analysis of HMGN knock out mice revealed that each HMGN protein has different physiological functions [21,49]. We therefore suggest, that functional integrity of the N-terminal NBD and the C-terminal regulatory domain will define the consequence of the interaction of individual HMGN proteins with the chromatin fiber, including competition with linker histones and transcription regulation. Further deciphering the relationship between the structural domains of individual proteins is required to understand the specificity and/or redundancy between the HMGNs on the level of individual cell and the whole organism.

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7. HMGN5 association with physiological and pathological processes

Aberrant expression of HMGN proteins is associated with developmental defects, hypersensitivity to stress and increased tumorigenic potential in mice [50]. Though the molecular role of HMGN5 is not yet understood, there are indications in the literature that suggest the potential involvement of HMGN5 in disease and in normal cellular functions (Table 1 and references therein). For example, microarray analysis and overexpression experiments identified HMGN5 as a factor that induces differentiation in mouse embryonic stem cells [51]. Upregulation of HMGN5 proteins was observed in prostate cancer [52] and squamous cell carcinoma in humans [53], adenocarcinoma in aging mice [54] and in the highly metastatic MDA-MB-435HM breast cancer cell line [55], suggesting a role for HMGN5 in tumorigenesis. The association of increased protein expression with cancer can be also predicted from the structure of the protein which is defined as intrinsically disordered [37].

The localization of HMGN5 gene on the X chromosome which contains a disproportionally high number of genes associated with mental functions and the late appearance of HMGN5 in evolution imply the possible association of the protein with behavior and cognition [56]. If demonstrated experimentally, this might shed light on the role of chromatin architecture in brain function. Interestingly, human ACRC protein, which has an HMGN5-like, bipartite structure composed of a positively charged C-terminal domain and negatively charged N-terminus containing a long stretch of acidic repeats, is also localized in proximity to the HMGN5 gene on the X chromosome [57].

8. Conclusions and perspectives

Understanding chromatin architecture and its regulation is crucial for deciphering the dynamics of biological processes, such as development and differentiation. It is progressively becoming clear that the regulation of chromatin architecture requires coordinative efforts of multiple proteins and that HMGN protein family is an integral part of this network. The discovery of HMGN5 extends the functional potential of HMGNs in the regulation of the structure of the chromatin fiber and emphasizes the variety of regulatory effects of different members of the same protein family on transcription. It is apparent now that even though HMGN5 binds to nucleosomes in a similar manner as other HMGNs, the outcome of this interaction on the global chromatin architecture is quite different. The unusually long and highly acidic tail which affects the interaction of linker histones with chromatin may confer to HMGN5 an added capacity to unfold higher order chromatin structures. Indeed, specific expression of HMGN5 in the placental giant cells which undergo endoreduplication without cell division suggests that the protein is required for the regulation of transcription from highly compact chromatin. The proposed dual mode of competition between HMGN5 and linker histones offers a new mechanism of the regulation of chromatin dynamics by HMGN proteins. Additional experiments are required to elucidate the molecular mechanism of this competition and its role in chromatin structure and function. We suggest that HMGN5 plays a significant role in maintaining the integrity of the chromatin fiber in cells and its aberrant expression is deleterious for the cell fate. Potential association of HMGN5 with cancer progression and brain activity makes this protein an attractive candidate for further research. However, similar to HMGN1, HMGN5 is probably not a “life or death” decision maker in the organism but rather a protein which affects the cellular phenotype by optimizing chromatin responses to the constantly changing environment.

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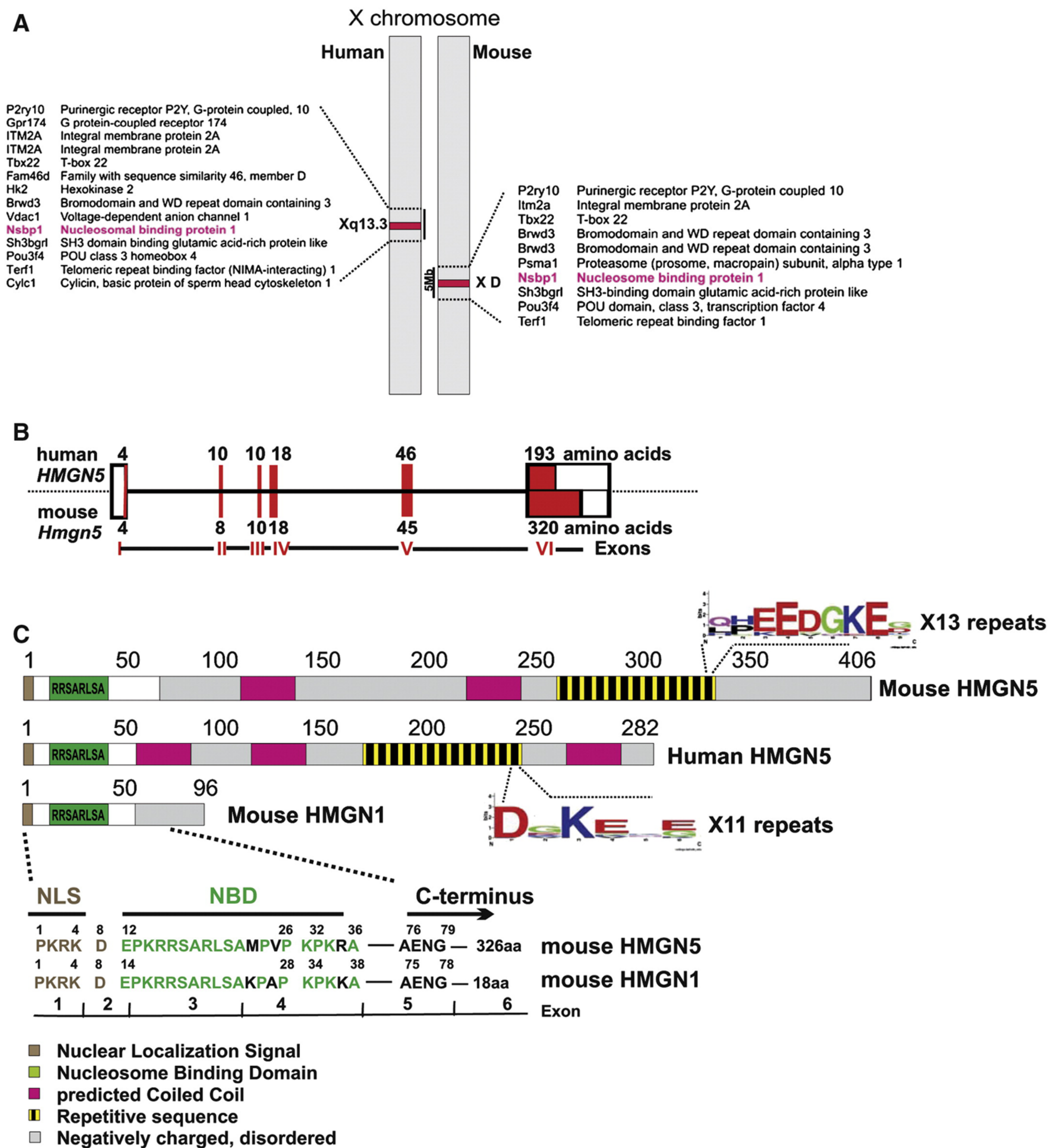

Figure 1. Comparison of human and mouse HMGN5

(A) Human and mouse HMGN5 genes are located at syntenic regions of the X-chromosome.
(B) Organization of the Hmgn5 gene. Exons are marked by red blocks and untranslated regions are marked by white boxes. The number of amino acids encoded by each exon is indicated.
(C) Schematic representation of structural domains of mouse and human HMGN5 and mouse HMGN1 proteins. Localization of each domain is related to the amino acid sequences of the proteins numbered on top of each scheme. Proteins are drawn to scale. Sequence alignment of the N-terminus of mouse HMGN5 and mouse HMGN1 and the corresponding gene organization are shown. Only amino acids from the highly similar regions are indicated. NLS – nuclear localization signal. NBD – nucleosome binding domain.

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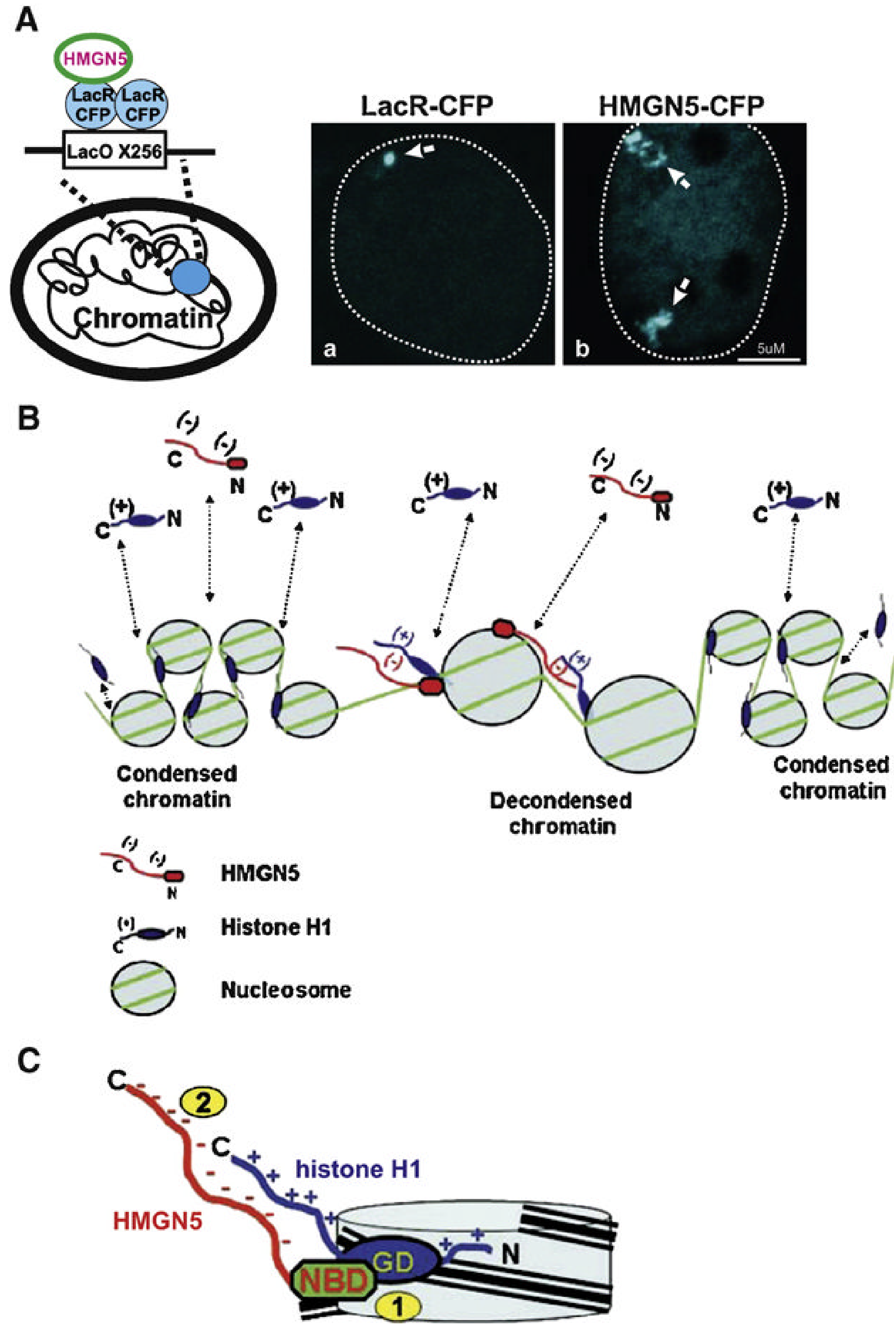

Figure 2. Model for HMGN5-mediated chromatin unfolding

(A) The interaction of HMGN5 with the nucleosomes decondenses a LacO chromatin array. Schematic drawing (left) demonstrates the strategy for tethering HMGN5 to the array by fusion to LacR-CFP. The condensed array, visualized by tethering LacR-CFP (a) appears as a compact dot. Tethering of the wild type HMGN5-CFP (b) to the array induced formation of a highly decondensed array as judged by formation of extended structures with irregular shape (arrows). Nucleus of the cells is marked by a broken line. (B) Effect of HMGN5 on chromatin compaction. In living cells H1 and HMGN5 bind dynamically to chromatin (dotted lines) randomly colliding on nucleosomes and counteracts the H1- induced stabilization of a compact chromatin structure. (C) The binding of HMGN5 to H1-containing nucleosome leads to two

molecular events: (1) competition of the NBD of HMGN5 with the Globular Domain (GD) of linker histone for the binding to the nucleosome and (2) juxtaposition and binding of the C-terminal domains of the proteins. Both events apparently contribute to the HMGN5-mediated chromatin unfolding. (B) and (C) are reproduced from [27].

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Table 1

HMGN5 association with pathological and physiological processes.

The protein is	Rodent HMGN5	Human HMGN5
Upregulated	• 4.7-fold in Ngn3-induced differentiation of mPACL20 cells into pancreatic islands [58] 1.6-fold in the androgen receptor KO mice [59] 1.6-fold in rat retinal ganglion cells cultured in glia-conditioned medium [60] 2-fold in MRL/lrp but not in NZB/W mouse model of lupus following IFN alpha stimulation of mononuclear cells [61] 2-fold in the embryo implantation site [39] In LPS-stimulated dendritic cells [62] In adult mouse uterus following the early exposure of mice to estrogenic compound diethylstilbestrol [54]	• By SATB1 in highly metastatic MDA-MB-231 breast cancer cells [63] 16-fold in a primary cutaneous squamous cell [53] 4.8-fold in MDA-MB-435HM (highly metastatic) compared to MDA-MB-435LM (low metastatic) breast cancer cells [55]
Down regulated	• 2.32-fold in the amyloid precursor protein TG mouse model of Alzheimer's disease [64]	
Other effects	• Promotes ES cells differentiation when overexpressed [51] Potentially associates with spontaneous ovarian granulose [65] Elevated expression is correlated with uterine adenocarcinoma in aging animals [54]	• Potentially associates with Martin–Probst deafness mental retardation syndrome, spinal muscular atrophy, coronary heart disease (OMIM database) Potentially associates with premature ovarian cancer [66] Potentially associates with androgen-dependent prostate cancer [52, 67]