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REVIEW

Yeast HMO1: Linker Histone Reinvented

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SUMMARY Eukaryotic genomes are packaged in chromatin. The higher-order organization of nucleosome core particles is controlled by the association of the intervening linker DNA with either the linker histone H1 or high mobility group box (HMGB) proteins. While H1 is thought to stabilize the nucleosome by preventing DNA unwrapping, the DNA bending imposed by HMGB may propagate to the nucleosome to destabilize chromatin. For metazoan H1, chromatin compaction requires its lysine-rich C-terminal domain, a domain that is buried between globular domains in the previously characterized yeast *Saccharomyces cerevisiae* linker histone Hho1p. Here, we discuss the functions of *S. cerevisiae* HMO1, an HMGB family protein unique in containing a terminal lysine-rich domain and in stabilizing genomic DNA. On ribosomal DNA (rDNA) and genes encoding ribosomal proteins, HMO1 appears to exert its role primarily by stabilizing nucleosome-free regions or "fragile" nucleosomes. During replication, HMO1 likewise appears to ensure low nucleosome density at DNA junctions associated with the DNA damage response or the need for topoisomerases to resolve catenanes. Notably, HMO1 shares with the mammalian

linker histone H1 the ability to stabilize chromatin, as evidenced by the absence of HMO1 creating a more dynamic chromatin environment that is more sensitive to nuclease digestion and in which chromatin-remodeling events associated with DNA double-strand break repair occur faster; such chromatin stabilization requires the lysine-rich extension of HMO1. Thus, HMO1 appears to have evolved a unique linker histone-like function involving the ability to stabilize both conventional nucleosome arrays as well as DNA regions characterized by low nucleosome density or the presence of noncanonical nucleosomes.

KEYWORDS HMGB proteins, linker histone, *Saccharomyces cerevisiae*, chromatin, nucleosome

INTRODUCTION

The DNA of all eukaryotic cells is tightly packaged into chromatin, a nucleoprotein complex consisting of DNA associated with histone and nonhistone proteins. The nucleosome is the fundamental unit of chromatin in which ~146 bp of DNA wrap around the histone octamer composed of two copies each of H2A, H2B, H3, and H4. The linker DNA connecting nucleosome core particles may associate with either linker histone H1 or nonhistone proteins such as the high mobility group box (HMGB) proteins, which often act in opposition to H1. In this review, we discuss the *Saccharomyces cerevisiae* HMGB protein HMO1, which is unique in encompassing features of both canonical HMGB proteins and linker histones. We consider its role in stabilizing nucleosome-free DNA and in modulating chromatin stability and dynamics during DNA repair and transcription.

Linker Histones

Nucleosome structure and organization. The static structure of the nucleosome core particle has been determined at high resolution, and the folding of a four-nucleosome array has also been reported (1, 2). The nucleosome core particle consists of a histone octamer composed of two H2A/H2B heterodimers and an $(H3/H4)_2$ heterotetramer about which ~146 bp of DNA is wrapped ~1.7 times in a left-handed supercoil (Fig. 1). Thanks to the identification of flexible DNA sequences that preferentially associate with core histones, it has been possible to achieve high-resolution structural information (3, 4). *In vitro*, nucleosome formation at a specific sequence is directed by intrinsic properties of the DNA, and it is nucleated by the association of the $(H3/H4)_2$ tetramer, which marks the initial point of DNA bending and therefore defines the dyad axis (Fig. 1A); the binding of $(H3/H4)_2$ is followed by the deposition of two H2A/H2B dimers (5–7). *In vivo*, nucleosome assembly is catalyzed by chaperones (8–10). The histone octamer makes numerous direct contacts to DNA, mostly in the DNA minor grooves, and the resulting DNA structure deviates significantly from the canonical *B*-form. Adjacent nucleosomes are connected by linker DNAs of various lengths to generate nucleosomal arrays (Fig. 2); the N-terminal tails of core histones extend away from the nucleosome core particle, and these positively charged extensions have been implicated in contacts to DNA and to neighboring nucleosomes.

Higher-order levels of organization in which nucleosomal arrays associate with other proteins remain poorly understood. Interactions between nucleosomes promote the folding of the nucleosomal array into a more compact 30-nm fiber, for example, by the interaction of the H4 N-terminal tail with an acidic patch formed at the H2A/H2B interface on a neighboring nucleosome (11). The linker histone H1 plays an indispensable role in stabilizing the 30-nm fiber in which nucleosomes are clustered tightly together, decreasing the internucleosomal distance and fixing the entry/exit angle of DNA (12–16). This compaction is affected by the nucleosomal repeat length, as the repeat length must be sufficient to accommodate H1 binding; for nucleosomal arrays with shorter repeat lengths, internucleosome interactions drive the folding of a more compact fiber that is less affected by linker histone binding (17). However, in proliferating cells, evidence of 30-nm fibers is lacking, and chromosome organization is instead thought to involve a zigzag geometry and long-range looping that is modulated by the

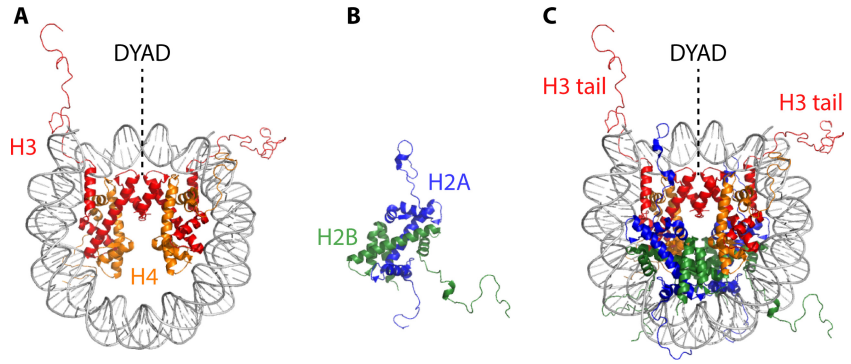

FIG 1 Assembly of the nucleosome core particle. (A) Association of the $(H3/H4)_2$ tetramer with DNA nucleates nucleosome assembly and defines the dyad axis. (B) H2A/H2B dimer. (C) Two H2A/H2B dimers are deposited to generate the nucleosome core particle. H3 N-terminal tails emerge near the DNA entry/exit points (based on data reported under PDB accession number 1KX5).

density of linker histones (18–22). This organization is thought to involve the formation of topologically associated domains by the formation of loops within higher-order chromatin structures; precisely how H1 mechanistically participates is unresolved.

Linker histone binding to the nucleosome. Histone H1 binds linker DNA where it enters and exits the nucleosome (Fig. 2) (23–26). Unlike core histones, which have residency times on a time scale of hours, linker histones are quite mobile, with residency times being measured in minutes (16, 27, 28). In mammalian cells, H1 is present at an average stoichiometry of ~0.8 molecules per nucleosome, with variations depending on the cell type, and in chicken, the ratio of linker histones to nucleosomes was estimated to be 1.3 histones per nucleosome (29). Metazoan linker histones have a tripartite structure. They interact with about 20 bp of DNA (either asymmetrically by preferentially binding one linker segment or by protecting 10 bp of entering and exiting DNA) to create the chromatosome, consisting of ~167 bp of DNA, the core histone octamer, and one molecule of H1. The ~40-amino-acid N-terminal domain is followed by the highly conserved central globular domain of ~80 amino acids and a long C-terminal domain (CTD) (~100 amino acids) (Fig. 3) (30). The globular domain adopts a winged-helix DNA binding motif (31); its interaction with DNA at the nucleosomal entry/exit points gives rise to protection of the additional ~20 bp (23, 32). The structure of the chicken linker histone H5 in complex with a nucleosome reveals the binding of the globular domain on the nucleosome dyad axis, interacting with both

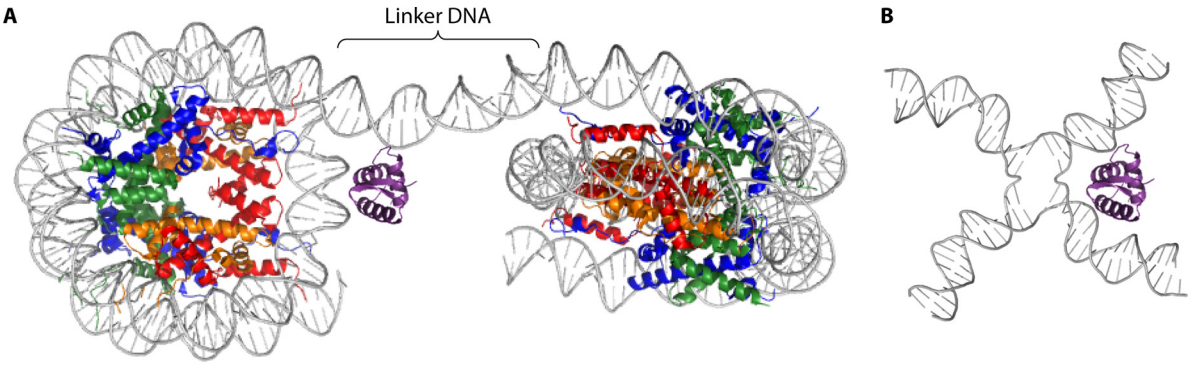

FIG 2 Histone H1 associates with linker DNA. (A) The globular domain of histone H1 (purple) binds the nucleosome at the dyad. The structure of a dinucleosome is depicted; the color coding for core histones is the same as that in Fig. 1. (B) Four-way junction DNA mimics the DNA configuration at the nucleosome dyad, perhaps explaining the preferred binding of H1 to such junctions. The dinucleosome represents the asymmetric unit in the structure of a tetranucleosome with one linker DNA trimmed for clarity (PDB accession number 1ZBB) (2). The H1 globular domain and its localization relative to the dyad are based on the structure of the chicken H5 globular domain in complex with a nucleosome (PDB accession number 4QLC) (33). The representation of the four-way junction is based on data reported under PDB accession number 3CRX.

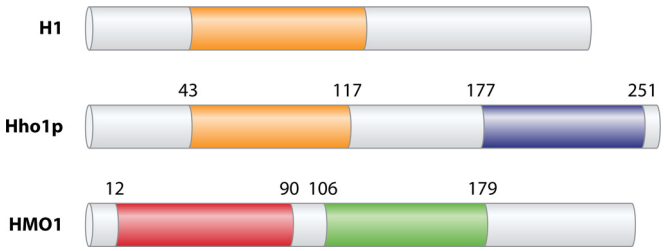

FIG 3 Domain organization of H1, Hho1p, and HMO1. Metazoan H1 typically contains an ~40-amino-acid N-terminal extension, followed by a globular domain of ~80 amino acids (orange) and a long CTD characterized by S/TPXK-like repeats. Hho1p contains a lysine-rich N-terminal segment followed by a globular domain with similarity to that of H1 (orange). Another lysine-rich segment connects this globular domain to the second globular domain (purple). HMO1 contains box A (red), which has little similarity to consensus HMG domains, followed by a lysine-rich linker, the box B domain (green), and a lysine-rich CTD. Mammalian HMGB proteins have a domain organization similar to that of HMO1, except that the CTD is acidic.

DNA linkers, whereas the *Drosophila melanogaster* linker histone H1 binds off-dyad (32, 33). Experiments involving site-directed mutagenesis followed by determination of binding to nucleosomes in living cells have likewise led to the conclusion that the globular domains of different mouse H1 isoforms use different interaction surfaces for contacts to chromatin (34, 35). This suggests that interactions with linker histones in different binding modes might differentially control higher-order chromatin organization. In general, linker histones bind preferentially to four-way DNA junctions compared to linear DNA (36), and binding to the nucleosome at the DNA entry/exit points is thought to reflect this preference for a specific DNA geometry (Fig. 2).

Notably, the regions flanking the globular domain, particularly the lysine-rich CTD, are required for the formation of higher-order structures (Fig. 4) (37, 38). The low-complexity sequence of the CTD, which includes ~40% lysine and a significant content of alanine and proline, results in the domain remaining unstructured in aqueous solution due to charge repulsion but acquiring a kinked-helix conformation when bound to DNA (39, 40). Interactions with the CTD promote the formation of higher-order chromatin structures as well as increase the residence time (23, 37, 41). Modeling suggests that a highly charged CTD compacts chromatin more effectively, resulting in silencing, whereas less-charged CTDs promote a chromatin folding in which the genome is more accessible (42). The N terminus, which is also unstructured, affects positioning and DNA binding affinity (43, 44). While specific H1 binding modes may be

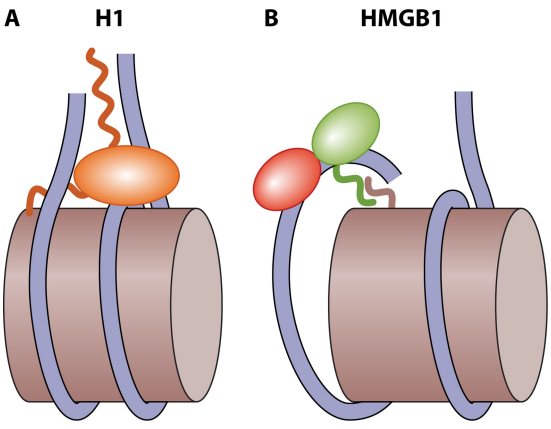

FIG 4 Proposed interaction of H1 and HMGB proteins with nucleosomes. (A) H1 binds near the dyad such that the CTD mainly contacts one linker segment; this creates a stem-like structure that stabilizes the nucleosome core (32, 38). (B) For HMGB, interactions between the acidic CTD and the H3 N-terminal tail that exits near the dyad promote binding of HMGB to DNA; the DNA bending and underwinding induced may propagate to the nucleosome core to promote unwrapping or access to other factors (118, 119).

distinct for different H1 isoforms, current data support a general mode of binding in which the H1 globular domain binds near the dyad axis, with the CTD mainly contacting one linker DNA such that linker DNA is organized into a stem-like structure (Fig. 4) (32, 38). In this configuration, one H1 has been proposed to link three nucleosomes and to prevent the association of additional H1 protomers, likely due to electrostatic repulsion.

Diversity of linker histones. The H1 family of linker histones is the most divergent class of histone proteins (45). For example, while the sequence of core histone H4 is 92% identical between yeasts and humans, the level of sequence identity between human H1 and the yeast linker histone Hho1p is only 31%. In addition, there are multiple different H1 subtypes in most eukaryotes (46). Some are constitutively expressed in all cells, while others are developmentally regulated, restricted to specific cell types, or induced at certain stages of differentiation. Covalent modifications contribute further to functional diversity (14–16, 47). Although the sequence of the winged-helix motif is relatively well conserved, the CTDs are extremely variable in both length and amino acid composition. Considering the role of the CTD in folding of nucleosomal arrays, different H1 isoforms are likely to exert different effects on chromatin organization. That the lysine-rich CTD is key to the organization of genomic DNA is reflected in euglenozoan protists, such as the kinetoplastids, which possess small linker histones that lack the winged-helix motif entirely and are similar to the basic CTD of metazoan histone H1 (48), although the amino acid composition may differ from that of the metazoan proteins. Such single-domain H1 proteins likely compact DNA by mechanisms that are distinct from those employed by metazoan H1. In contrast, the *Gallus gallus* (chicken) erythrocyte linker histone H5 shares greater sequence homology to human histone H1.0 (66% identity in comparisons of the complete sequences), with the CTD being more divergent (49).

Yeast linker histone Hho1p. In contrast to higher eukaryotes, less is known about linker histone function in *S. cerevisiae*. Sequencing of the yeast genome showed the existence of an unusual linker histone H1, named Hho1p, characterized by having two globular domains, one of which has significant homology to the globular domain of metazoan H1 (Fig. 3) (50). A short basic tail precedes the H1-like globular domain, and the second globular domain follows a lysine-rich linker. No other linker histones that contain two globular domains have been reported. While the first globular domain closely resembles the winged-helix-turn-helix motif characteristic of metazoan H1, the second globular domain is unstructured under physiological conditions but adopts a winged-helix fold in the presence of high concentrations of tetrahedral anions (51). Only the first globular domain can associate with nucleosomes to protect additional DNA from nuclease digestion *in vitro*, whereas the second domain exhibits the greatest affinity for four-way junction DNA (52, 53). Four-way junction DNA may mimic the DNA conformation at nucleosomal entry/exit points (Fig. 2), and the ability of Hho1p to bind two four-way junction structures simultaneously has been reported, raising the possibility that Hho1p may bridge two adjacent nucleosomes (54); however, direct evidence for such binding has not been demonstrated.

Micrococcal nuclease (MNase) digests accessible linker DNA to produce DNA fragments corresponding to the chromatosome, whereas the digestion of nucleosomal arrays depleted of H1 is faster and generates shorter ~146-bp fragments corresponding to the nucleosome core particle (55). In contrast to the nuclease sensitivity that results from eliminating H1, the absence of Hho1p does not result in a significant reorganization of nucleosomes or a change in the chromatin structure during vegetative growth, perhaps due to the absence of a terminal lysine-rich domain (50, 56). In addition, the stoichiometry of Hho1p to nucleosomes is lower than that of metazoan H1; estimates of cellular concentrations of Hho1p suggested a ratio of 1 Hho1p molecule per 37 nucleosomes (or ~2,000 molecules/haploid cell) (57), a number that is in good agreement with a more recent estimate of the cellular protein content (~2,600 molecules/cell) using a proteomics approach (58). A separate study indicated a ratio of 1 Hho1p molecule per 4 nucleosomes by comparison to the cellular content of histone

H2A (59); however, since core histones are produced in excess over the quantities required for nucleosome assembly during S phase (60), this number may be overestimated. The proposed binding mode for Hho1p, in which its globular domains simultaneously engage adjacent nucleosomes, would be expected to generate a different type of nucleosome compaction from that of H1; in comparison, one H1 molecule has been proposed to link three nucleosomes to generate a zigzag pattern. Different binding modes would be consistent with differential sensitivities to MNase of H1- or Hho1p-containing chromatin (38, 54).

Genome-wide, Hho1p binding was shown to be variable and to be concentrated at ribosomal DNA (rDNA), where it has been implicated in repressing the expression of RNA polymerase II (Pol II)-transcribed reporter genes embedded in the rDNA, suggesting a role in rDNA compaction (61). A general role for Hho1p in the formation of DNA loops and for DNA compaction during stationary phase was also reported (62, 63). In contrast, Hho1p has also been demonstrated to prevent the establishment of silent chromatin, perhaps by modifying the barriers that separate transcriptionally active chromatin from heterochromatin (57, 63–65). Thus, both H1 and Hho1p have been implicated in long-range DNA looping and DNA compaction; however, the molecular mechanisms by which these proteins exert such functions are likely to differ.

Chromatin represses transcription. In general, transcriptional repression correlates with chromatin condensation. Even nucleosomal arrays are repressive to transcription, as nucleosomes prevent transcription factors from accessing their cognate DNA. Promoters are therefore typically depleted of nucleosomes compared to the transcribed regions. Such nucleosome-free regions are found just upstream of the transcriptional start site, while the +1 nucleosome, which is found downstream of the start site, is localized strongly to this position. In yeast, nucleosome-free regions are typically maintained by transcription factors (66, 67).

Genome-wide profiling has demonstrated not only an absence of nucleosomes from active gene promoters in human cells but also a more extensive absence of linker histones, both upstream and downstream of the transcriptional start site (68). An early instructive example of transcriptional repression by H1 in *Xenopus laevis* shows that reduced H1 expression leads to the upregulation of 5S rRNA expression (69). More recent studies have suggested gene-specific transcriptional regulation by H1 as opposed to global effects and that H1 subtypes have an uneven distribution across the genome (70, 71). For example, chromatin immunoprecipitation (ChIP) showed a relative depletion of human H1.2 and H1.4 in actively transcribed chromatin, whereas all somatic subtypes were detected in heterochromatin (72). Consistent with this observation, H1.2 was reported to be overexpressed in cancer cells, where it is recruited to target genes by association with trimethylated H3 lysine 27 (H3K27me3) and contributes to the establishment of silent chromatin by a mechanism that requires its CTD (73). In heterochromatin, methylated H1 is implicated in the recruitment of factors such as heterochromatin protein 1 (HP1) (74, 75). Consistent with the ability of H1 to organize linker DNA into a stem-like structure (Fig. 4), H1 has been proposed to repress transcription by limiting nucleosome unwrapping as opposed to physically blocking transcription factor binding (76). This is consistent with genome-wide analyses that point to extensive H1 displacement in transcriptionally active genes (68, 77). Such a displacement may be aided by chaperones (78).

Chromatin represses DNA repair. Genome integrity is continuously challenged by both endogenous and environmental agents that induce DNA damage. Such damage occurs in the context of chromatin, and higher-order chromatin structures are generally repressive for DNA repair. The "access-repair-restore" model describes sequential events involved in DNA repair in terms of detection of the lesion, chromatin remodeling to allow access to the repair machinery, the actual repair event, and, finally, restoration of the original chromatin state (79, 80). In this scenario, chromatin is viewed as a barrier that needs to be dismantled for DNA repair to proceed. However, the picture is more complex, and emerging evidence has pointed to a role for the nucleosome in recruiting DNA repair proteins (81).

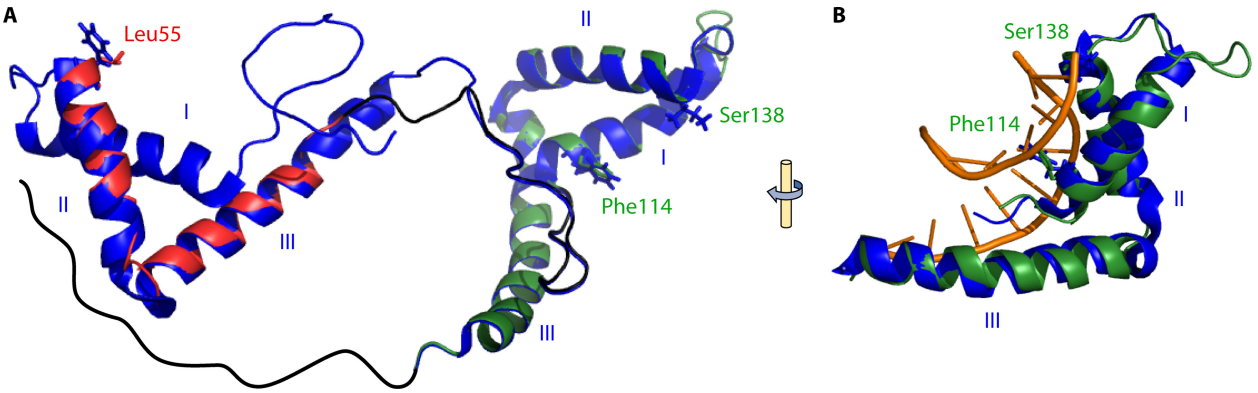

FIG 5 Model of HMO1 and its interaction with DNA. (A) HMO1 was modeled by using SwissModel in the automated mode, using human HMGB1 (PDB accession number 2YRQ) as the template. HMGB1 is shown in blue, and the HMO1 model is overlaid with box A and box B domains in red and green, respectively. Predicted HMO1-intercalating residues Leu55, from box A, and Phe114, from box B, are shown in a stick representation. Ser138 is located in the position occupied by the DNA-intercalating Ile residue in HMGB1 box B. Helices are identified with roman numerals. The HMO1 C-terminal extension (black) is inferred to interact with box A. (B) HMGB1 box B (blue) overlaid with HMO1 box B (green), showing the interaction of helix III in the DNA minor groove and the intercalation of Phe between DNA bases. HMGB1 box B DNA is based on data reported under PDB accession number 2GZK.

Among the various DNA lesions, double-strand breaks (DSBs) are particularly genotoxic, and continuous DNA damage without efficient DSB repair may result in tumorigenesis and ageing. The primary DSB repair pathways include homologous recombination (HR) and nonhomologous end joining (NHEJ). Homologous recombination relies on DNA homology between sister chromatids and precisely repairs DSBs, while NHEJ is a more error-prone process that uses no or very limited sequence homology to rejoin two DNA ends (82). DNA repair proteins such as Ku and Rad51p play a critical role in DSB repair. Rad51p promotes homologous recombination to repair DSB lesions; however, the chromatosome inhibits homologous pairing. To overcome this barrier and to aid Rad51p-mediated homologous pairing, Rad54p, a member of the ATP-dependent nucleosome remodeling factor family, is required in both yeast and metazoans (83, 84). The linker histone functions as a negative regulator to suppress inappropriate DNA recombination, which may cause chromosomal aberrations. In *S. cerevisiae*, Hho1p was also reported to suppress homologous recombination (59, 85). To efficiently repair DSBs by Rad51p- and Rad54p-mediated homologous recombination, the linker histone H1 is evicted by a histone chaperone, Nap1p, from a reconstituted nucleosome array, suggesting that eviction of H1 promotes repair by homologous recombination (86). In contrast, Ku, which is integral to DSB repair by nonhomologous end joining, has been reported to readily displace human H1 from DNA ends (87). In human cells, ubiquitylation of H1 at DSB sites was also recently implicated in the recruitment of repair factors, adding to the collection of histone marks that contribute to repair events (88).

High Mobility Group Proteins

In eukaryotes, high mobility group (HMG) proteins are abundant nuclear proteins that make up a significant fraction of DNA binding nonhistone proteins. The HMGB protein family is the largest family of HMG proteins and a major nucleosome-binding constituent of the metazoan nucleus (89). In mammalian cells, the nucleus contains approximately 1 HMGB protein for every 10 nucleosomes (90). In addition to roles in DNA-dependent events, HMGB proteins sense cellular stress and function as extracellular cytokines, contributing to inflammatory and immune responses (91).

DNA binding by HMGB proteins. The HMGB subfamily is divided into two classes, sequence-specific transcription factors that are expressed in a few cells and non-sequence-specific chromatin-associated proteins, which are abundant constituents of all eukaryotic nuclei. Transcription factors such as LEF-1 and SRY usually contain a single 80-amino-acid HMG box in which three α-helices create an L-shaped motif (Fig. 5B) (92). Most non-sequence-specific chromatin-associated HMGB proteins, e.g., mammalian HMGB1 to -4, possess two HMGB domains and bind preferentially to non-*B*-form DNA

structures such as four-way junctions and DNA modified by the anticancer agent cisplatin (93, 94). Exceptions have been described, for example, in *S. cerevisiae* and *Drosophila melanogaster*, which encode single HMG box proteins (NHP6A/B and HMGD, respectively) that bind DNA without a sequence preference (95, 96).

The HMG box serves as the primary site of binding to DNA and chromatin. The interaction of HMGB proteins with DNA is very dynamic; HMGB proteins bind transiently to *B*-form DNA and bend their DNA targets, and the mode of interaction of HMGB proteins with chromatin has therefore been characterized as a "hit and run" (93, 97). The energy required for DNA bending is derived from the extensive contacts of HMG boxes with the minor groove of DNA. Since the energetic cost of bending DNA is decreased in distorted or prebent DNA, HMGB1 proteins associate with such distorted DNA structures with high affinity (93, 98–100). The functional consequences of HMGB1 binding to damaged DNA have been alternately suggested to be a shielding of the lesion from the repair machinery or enhanced recognition of the damaged site (99, 101, 102). For example, HMGB1 has been reported to sensitize cells to cisplatin by impeding repair, perhaps influenced by the cellular redox state (103, 104); conversely, human HMGB1 has been reported to facilitate nucleotide excision repair (NER) by recruiting the NER protein XPA to interstrand cross-links (105). Interestingly, the recruitment of XPA to nondamaged sites was increased in HMGB1-depleted cells, suggesting that HMGB1 not only promotes NER but also facilitates the specificity of XPA-mediated damage recognition.

Structure of HMGB proteins. The canonical HMGB proteins have a molecular mass of ~25 kDa, containing two similar HMG domains, boxes A and B, and a C-terminal tail of ~30 acidic amino acids (Fig. 3). Despite their similarity, box A differs from box B in the relative orientations of helices I and II and in the trajectory of the helix I-helix II loop, and the two domains have distinct electrostatic surface potentials in their DNA binding regions. The concave sides of both domains bind in the minor groove of DNA by using van der Waals and electrostatic interactions to induce a bend toward the major groove. The A domain has a greater preference for distorted DNA (106, 107), whereas the B domain binds less selectively to distorted DNA structures but can introduce an approximately right-angled bend into linear DNA (108). Partial DNA intercalation of hydrophobic residues located toward the N terminus of helices I and II introduces a kink into the bound DNA and thus enhances the bend associated with a widening of the minor groove (Fig. 5B). The bends induced by either domain likely reinforce each other (109, 110). For HMGB1, acetylation of lysine residues in the box A domain occurs *in vivo*, and it has been reported that substitution of these lysine residues compromises the preferred binding to both four-way junction DNA and constrained minicircles (107).

An important feature of HMGB1 and HMGB2 is the presence of a long acidic C-terminal "tail" consisting of ~30 acidic residues (HMG1) or ~20 acidic residues (HMG2). The acidic tail interacts primarily with box B but functions to decrease the DNA-binding affinity of both domains (94, 111, 112). Furthermore, the tail is required for preferential binding to DNA minicircles relative to linear DNA (113). A dynamic assembly in which the acidic tail transiently brings the two HMG box domains together has been proposed. On account of the ability to bend DNA, HMGB proteins are generally referred to as architectural, creating nucleoprotein complexes in which the modified DNA structure promotes the association of additional proteins. In this capacity, HMGB proteins participate in numerous DNA-dependent functions, ranging from DNA replication to gene transcription.

Binding of HMGB proteins to nucleosomes. Consistent with the preferred binding to four-way junction DNA *in vitro* in the open-square conformation that is preferred in the absence of divalent cations (114), HMGB proteins bind nucleosomes at DNA entry/exit points (Fig. 2). The DNA bending and underwinding that result from HMGB1 binding are transmitted to the nucleosome core (Fig. 4). This may affect contacts between DNA and core histones and prime the nucleosome core for binding of transcription factors or chromatin-remodeling complexes; thus, HMGB binding is generally associated with more dynamic chromatin and facilitated transcription. HMGB1 binding in the vicinity of the DNA entry/exit points on the nucleosome may be facilitated

by interactions between its acidic tail and the N-terminal tail of histone H3, which exits near the DNA entry/exit points of the nucleosome and contacts the linker DNA (Fig. 1) (112, 115–119). Binding of the HMG boxes to DNA frees the acidic tail from intramolecular interactions with the DNA binding surfaces, allowing it to interact with H3. A predicted consequence of this interaction is enhanced DNA binding by HMGB1 (120).

Although mammalian H1 has a high affinity for reconstituted dinucleosomes compared to HMGB1, the binding of HMGB1 displaces the linker histone, perhaps aided by its preferred binding to constrained DNA conformations (121–124). *In vitro*, an interaction between the acidic tail of HMGB1 and the linker histone H1 has also been reported, suggesting that interactions with H1 may increase the DNA binding affinity of HMGB1 by preventing interactions between the acidic tail and the HMG domains, thereby facilitating the replacement of H1 with HMGB1 (125). This enhanced binding of HMGB1 in turn affects chromatin remodeling, for example, by facilitating the binding of the ISWI-containing remodeling factors ACF and CHRAC to chromatin (115).

YEAST HMGB PROTEIN HMO1

S. cerevisiae expresses several HMGB proteins, of which HMO1 and HMO2 contain two globular HMG-like domains. HMO2 (also known as NHP10), which is unique in exhibiting a preferred binding to DNA ends, is a component of the INO80 chromatin-remodeling complex that is recruited to DNA damage sites (126, 127). HMO1 was first identified by its copurification with an unidentified DNA helicase (128). HMO1 has also been identified in closely related species such as *Saccharomyces kluyveri* (129). In addition, an HMO1 counterpart is encoded in the *Schizosaccharomyces pombe* genome (130). According to the *Saccharomyces* Genome Database (131), HMO1 has been reported to exhibit physical or genetic interactions with a total of 290 genes or gene products. HMO1 is quite abundant, with reported estimates of 19,000 to 25,000 molecules/cell, corresponding to 1 HMO1 molecule per 3 to 4 nucleosomes (58, 60).

HMO1 Domain Organization

HMO1 has two globular domains, named box A and box B, of about 80 amino acids each, similarly to mammalian HMGB (Fig. 1). Box A, which has only limited similarity to consensus HMG domains, functions as a dimerization domain; it has a low affinity for DNA but exhibits some structural specificity, including preferred binding to four-way junction DNA, whereas canonical box B has a higher affinity for DNA but a lower structural specificity (130, 132). The HMO1 box A domain contributes to DNA bending; in contrast, the box B domain contributes most of the DNA binding affinity but fails to bend linear DNA (133). There is no high-resolution structural information available for HMO1; however, a structure-based model predicts that both box A and box B domains adopt the HMG fold (Fig. 5A). Alignment with the human HMGB1 that was used as a template for modeling predicts that Leu55 at the end of helix II corresponds to the HMGB1 box A-intercalating residue (Phe in HMGB1); due to poor sequence conservation at the start of box A, it cannot be predicted with confidence if a potential intercalating residue is present at the end of helix I (Phe at the end of HMGB1 box A helix II is the only intercalating residue in this domain). For HMGB1 box B, Phe and Ile are the DNA-intercalating residues found at the ends of helices I and II, respectively; the corresponding residues in HMO1 box B are Phe114 and Ser138 (which is not predicted to intercalate between DNA bases).

In addition to the A and B domains, HMO1 has a C-terminal domain that is characterized by a stretch of basic amino acids; this is in marked contrast to the mammalian HMGB protein, in which the C-terminal extension is acidic. Deletion of the lysine-rich extension does not reduce the affinity for linear DNA, arguing against a direct interaction between the CTD and this type of DNA substrate. Instead, interactions between box A and the C-terminal extension were reported to induce a conformation that is required for in-phase DNA bending *in vitro* (133, 134).

Deletion of the *HMO1* gene is not lethal but results in a severe growth defect and reduced plasmid stability (128, 135). Inactivation of *HMO1* is synthetically lethal with

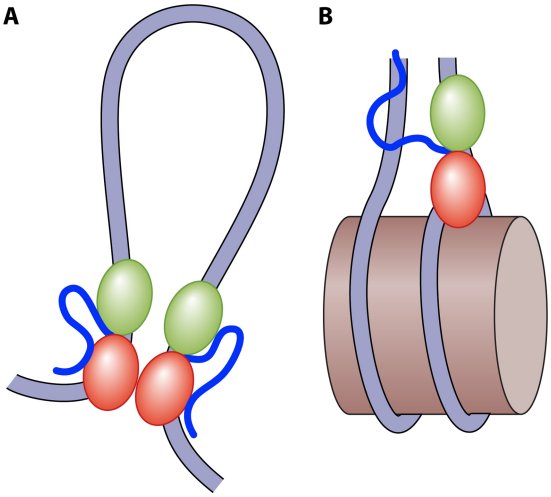

FIG 6 HMO1-mediated stabilization of genomic DNA. (A) On nucleosome-free DNA, HMO1 promotes the formation of loops and bridges that depend on the dimerization of the box A domains (151). Such topological domains may also be mediated by the concerted action of HMO1 and Top2 (195). (B) Both HMO1 and HMO1 deleted for its C-terminal domain can compete with human H1 for binding to nucleosomes, suggesting that HMO1 and H1 binding sites at least partially overlap (198). A possible nucleosome-stabilizing binding mode for HMO1 is illustrated, in which the structure-specific box A domain binds near the dyad and DNA bending by HMO1 is prevented due to the lysine-rich CTD contacting linker DNA.

*fpr1*Δ, a deletion that also results in a plasmid loss phenotype; *FPR1* encodes the peptidyl-prolyl *cis-trans* isomerase FKBP12, and overproduction of HMO1 in cells deleted for *FPR1* is toxic. FKBP12 disrupts the self-association of HMO1, suggesting that toxicity could be due to either the uncontrolled accumulation of HMO1 at certain target DNA sites or the sequestering of unbound HMO1 (136). FKBP12 is otherwise best known as the receptor for the immunosuppressive drugs FK506 and rapamycin, and binding to either drug is toxic due to the inhibition of signal transduction (137).

HMO1 Is a Component of the Pol I Transcription Machinery

Transcription of rRNA genes by Pol I has been suggested to be the rate-limiting step in ribosome production (138, 139). Intricate networks adapt rRNA production to metabolic rates, as ribosome production must keep up with cellular demands. The synthesis of rRNA, which accounts for at least 60% of the total transcriptional activity during normal growth, is in most cases thought to be regulated based on the control of active genes as opposed to epigenetic mechanisms that change the ratio of active to silenced genes (140, 141). Distinct nuclear compartments, the nucleoli, form around the rDNA, and nucleolar structure and cell cycle progression are dependent on rDNA transcription (142, 143).

HMO1 associates with rDNA. In *S. cerevisiae*, ~150 rDNA repeats are arranged head to tail on chromosome XII. Each repeat encodes 35S rRNA synthesized by RNA Pol I and the Pol III-transcribed 5S rRNA. In exponentially growing cells, more than half of the rDNA is transcriptionally silenced (144–147). The remaining fraction constitutes active rRNA genes that are largely depleted of nucleosomes but instead loaded with RNA Pol I and HMO1, with HMO1 stabilizing the open chromatin state in the absence of RNA Pol I transcription (145, 148). While an initial analysis suggested that HMO1 was bound throughout the rDNA (149), a more stringent approach revealed preferred HMO1 binding to Pol I-transcribed regions of the rDNA and that HMO1 remained bound in the absence of Pol I (148, 150). A mechanism by which HMO1 may secure a nucleosome-free region of rDNA involves the dimerization of HMO1 through its box A domains to stabilize DNA bridges and loops (Fig. 6) (151). However, the looped DNA structure formed by HMO1 is dynamic and is predicted to be easily disrupted by the force generated by a transcribing RNA polymerase (151). HMO1 has also been implicated in

the resumption of RNA Pol I transcription elongation and reopening of rDNA chromatin after DNA repair; UV light-induced DNA lesions block transcription and lead to a special chromatin structure at the rDNA locus characterized by the dissociation of RNA Pol I and loading of histones downstream of the lesion but retention of HMO1 (152).

Upon nutrient limitation, rDNA transcription is downregulated, and this correlates with a reduction in nucleolar size, a process that is dependent on condensins and involves a compaction of the rDNA (153). It was recently reported that HMO1 is also involved in such a contraction of the nucleolus and that its binding is increased across the 35S rRNA gene in response to starvation (154). As noted above, several previous studies have shown HMO1 binding either across the rDNA or with a preferred association with transcribed regions, depending on method of detection, ChIP protocol, and normalization strategy (148–150, 155). In contrast, Wang et al. (154) reported limited HMO1 binding to 35S rRNA genes in log-phase cells (4 h of growth following inoculation of cultures) and an ~6-fold enrichment during nutrient limitation (24 h of growth); whether the failure to detect HMO1 binding in log-phase cells is due to variations in ChIP protocols or to the genetic background is not clear. The basis for these differences notwithstanding, the reported increase in HMO1 binding upon nutrient limitation would be consistent with a contribution of HMO1 to rDNA compaction under such conditions.

A functional equivalent of UBF. HMO1 preferentially associates with the transcribed region of the 35S rDNA locus, and it promotes rRNA production both as a component of the RNA Pol I transcription apparatus and by facilitating rRNA maturation (149, 150, 155, 156). Overproduction of HMO1 suppresses the severe growth phenotype caused by a deletion of the gene encoding Rpa49p, a conserved subunit of RNA Pol I and the homolog of human PAF53, and *rpa49*Δ-*hmo1*Δ double mutants are inviable, indicating that HMO1 is a component of the Pol I transcription machinery (156). Rrn3p is required for initiation by yeast RNA Pol I, and it is subsequently released during elongation in a process that requires Rpa49p and the presence of another transcribing RNA polymerase (157). The absence of Rpa49p also leads to a decreased density of transcribing RNA polymerases on a given gene, a consequence of which is compromised assembly of the nucleolus (158). The increased distance between transcribing polymerases in the *rpa49*Δ mutant would also be expected to result in a topological strain due to positive DNA supercoiling accumulating in front of a polymerase and negative supercoiling developing in its wake; consistent with an *rpa49*Δ mutant accumulating torsional stress, *rpa49*Δ is lethal when the type I topoisomerase Top3 is inactivated (156). Since HMO1 bends and loops DNA, it may counteract the torsional stress imposed by transcribing Pol I, thereby alleviating the *rpa49*Δ phenotype. Alternatively, or in addition, the absence of HMO1-mediated DNA looping on rDNA not associated with transcribing RNA polymerase may lead to nucleosome deposition that is inhibitory to transcription (145).

In mammals, RNA Pol I requires upstream binding factor (UBF) for initiation and elongation (159–161). UBF contains six HMG boxes and binds throughout the rRNA gene locus (162). However, yeast lacks UBF, and HMO1 has been proposed to be a functional analog of UBF and to be important for maximal Pol I transcription (156). Comparable functions of UBF and HMO1 are supported by the observation that both proteins are highly enriched in the nucleolus and localized throughout the transcribed rDNA region and that both proteins contain HMG domains that may promote DNA bending and DNA looping (149, 150, 162, 163). Further support for overlapping functions of HMO1 and UBF was provided by the observation that the expression of human UBF1 or *S. pombe* HMO1 also suppresses the *rpa49*Δ growth phenotype (130).

HMO1 Regulates Pol II Transcription

Ribosomal protein gene expression. In *S. cerevisiae*, the ribosome is made up of four rRNAs (5S, 5.8S, 18S, and 25S) and 79 ribosomal proteins (RPs) expressed from 138 genes (164). RP gene transcription constitutes up to 50% of RNA Pol II-mediated transcription, and it is coordinately regulated in response to environmental conditions (165). In prokaryotes, ensuring the production of stoichiometric levels of ribosomal

proteins is simple because RP genes form operons, whereas in eukaryotes, such regulation is more complicated, as each RP gene yields a monocistronic mRNA. A number of transcription factors have been reported to contribute to the regulation of RP gene activity, including Rap1p, which binds the majority of RP genes and forms nucleosome-free regions in target promoters (155, 166). HMO1 binds RP gene promoters with variable occupancy and has been implicated in preinitiation complex (PIC) assembly by covering a nucleosome-free region and in the recruitment of the transcription factor Fhl1p (forkhead like) (149, 150, 155, 167, 168).

Pol II transcription requires basal transcription factors, including TFIID, which contains the TATA box binding protein (TBP), and TBP-associated factors (TAFs). The TAF1 N-terminal domain (TAND) inhibits the binding of TBP to the TATA element (169); it has been reported that HMO1 interacts with TBP and TAND and that *HMO1* deletion decreases the transcription of TAND-dependent genes, suggesting that HMO1 prevents inhibitory TBP-TAND interactions. In addition, an interaction between HMO1 and TFIID was suggested by the observation that *HMO1* deletion causes an upstream shift in transcription start sites of genes under the control of HMO1-enriched promoters but not of genes under the control of promoters with limited HMO1 occupancy (168). This shift in the transcriptional start site was subsequently linked to the ability of HMO1 to mask a nucleosome-free region to prevent inappropriate PIC assembly (167). This nucleosome-free region was later reported to exhibit sensitivity to micrococcal nuclease and to contain unstable or "fragile" nucleosomes, perhaps rendered unstable through the action of the essential multifunctional transcription factor Rap1p (170).

Fhl1p is a transcription factor with sequence similarity to the forkhead (FH) winged-helix DNA binding domain. On RP genes, Fhl1p has been reported to remain bound and to recruit either the coactivator Ifh1p (interacts with forkhead) or the corepressor Crf1p. During vigorous growth, Fhl1p and Rap1p recruit Ifh1p, which results in maximal transcription (171–173). In addition, Sfp1p (split finger protein) has been reported to be required for maximal transcription from RP promoters (174). During stress and nutrient starvation, Ifh1p dissociates from RP promoters, and Fhl1p recruits Crf1p, while Sfp1p translocates to the cytoplasm, events that lead to the downregulation of RP gene transcription (171, 172, 174, 175). Dissociation of HMO1 was also reported under conditions of RP gene repression, leading to an upstream shift of the +1 nucleosome, suggesting that HMO1 is important for the placement of the +1 nucleosomes in either a repressive or an active position (176).

Regulation of non-RP gene transcription. Little is known about the role of HMO1 in the regulation of Pol II-transcribed genes other than the RP genes. Excess HMO1 represses the *HMO1* promoter; however, the underlying mechanism is unknown (135). Given the self-association of HMO1, it is tempting to speculate that excess HMO1 promotes an accretion of HMO1 on the *HMO1* promoter that adversely affects the binding of either transcription factors or RNA Pol II. In contrast, the absence of HMO1 results in the reduced transcription of the *MAT***a** gene, a locus to which HMO1 also binds directly, suggesting that the effect may be direct (177).

Regulation of gene transcription involves ATP-dependent chromatin-remodeling complexes that either slide or evict nucleosomes or alter their composition (178). The conserved SWI/SNF complex, for instance, is critical for the modulation of gene expression during a variety of cellular processes. Among the HMGB proteins, HMO1 and NHP6A/B stimulate the sliding activity of SWI/SNF, but only HMO1 promotes SWI/SNF binding to the nucleosome, histone octamer transfer, and exposure of nucleosomal DNA. Notably, the stimulatory effect requires the HMO1 CTD and the presence of linker DNA, as no binding of HMO1 to nucleosomes devoid of linker DNA could be detected (179). Based on these observations, HMO1 appears to recruit SWI/SNF to nucleosomes by a mechanism that requires changes in the DNA topology or organization of the nucleosomal array.

HMO1 Coordinates Responses to TORC1 Signaling

Control of rRNA synthesis. Cell growth requires ribosome biogenesis, and several signaling pathways participate by sensing environmental cues such as stress, nutrient

limitation, or growth factors. The serine/threonine protein kinase TOR (target of rapamycin) is a central regulator of ribosome biogenesis. Both yeast and mammalian cells have two functionally different TOR complexes, TOR complex 1 (TORC1) and TOR complex 2 (TORC2); TORC1 is stimulated by amino acid sufficiency, and it responds to rapamycin to generate responses akin to those elicited by starvation and environmental stress such as hypoxia and DNA damage to reduce cell growth (180, 181). TORC2 is rapamycin insensitive and controls the organization of the actin cytoskeleton (181–183).

In addition to targeting the translation machinery, TORC1 promotes the transcription of genes whose products are involved in ribosome biogenesis. TOR kinase is recruited directly to rRNA gene promoters in a nutrient-dependent fashion; in the presence of rapamycin, rDNA becomes condensed (and the nucleolus contracts by a mechanism in which HMO1 participates, as noted above), and a rapid delocalization of RNA Pol I from the nucleolus is observed, along with TOR kinase exiting the nucleus (184, 185). Rapamycin treatment has also been reported to result in the dissociation of HMO1 from rDNA, and TORC1-dependent repression of rDNA transcription is reduced in the absence of HMO1, suggesting a role for HMO1 in communicating TORC1 activity (150). Nutrient limitation is a condition expected to inhibit TORC1; as noted above, a recent study suggested that nutrient limitation (achieved by growing yeast cells for 24 h following inoculation) resulted in increased HMO1 binding to rDNA and to nucleolar contraction (154). It would be instructive to determine TORC1 activity after 24 h of growth compared to the more extensive depletion of nutrients and to investigate if increased HMO1 binding to rDNA may be a transient phenomenon. Prolonged exposure to rapamycin results in the downregulation of the *HMO1* promoter and reduced cellular HMO1 levels (135, 186), perhaps rationalizing an initial increase in the HMO1 association with rDNA upon the inhibition of TORC1 signaling, followed by its dissociation as cellular levels decrease.

TORC1 signaling has also been reported to control histone H3 lysine 56 acetylation (H3K56ac), with H3K56ac promoting the binding of HMO1 (187). H3K56ac is generally associated with the disassembly of nucleosomes by disrupting interactions between H3 and DNA where DNA enters and exits the nucleosome (188). Accordingly, H3K56ac may promote a nucleosome-free region in rDNA to which HMO1 is recruited, while dissociation of HMO1 from rDNA upon the inhibition of TORC1 may be linked to reduced H3K56ac.

Control of RP gene expression. TORC1 also controls RP gene expression. For example, inhibition of TORC1 signaling by rapamycin releases the histone acetyltransferase ESA1 from RP gene promoters and leads to histone H4 deacetylation (184). Several transcription factors have been implicated in the regulation of RP gene activity. At least part of the rapamycin-mediated downregulation of RP gene activity can be attributed to the accumulation of Crf1p in the nucleus, an event that is triggered by its phosphorylation in response to nutrient limitation (171, 173, 189, 190). TORC1 signaling also leads to the accumulation of Sfp1p in the nucleus (174, 175). Under optimal growth conditions, Sfp1p binds RP gene promoters and activates transcription, perhaps via an interaction with Fhl1p and Ifh1p, while inhibition of TORC1 signaling results in the release of Sfp1p and its exit from the nucleus (171, 174, 175). HMO1 has also been reported to dissociate from some RP gene promoters in response to rapamycin, and reduced TORC1-dependent repression of RP gene expression was observed in *hmo1*Δ cells (150). Notably, the expression of *HMO1* is also repressed upon the inhibition of TORC1 signaling in an HMO1- and Fhl1p-dependent fashion, suggesting a mechanism comparable to that proposed for the TORC1-mediated repression of RP genes (135). Clearly, HMO1 is linked to the TORC1 signaling pathway and may be involved in coordinating rDNA transcription and RP gene expression (150, 191).

HMO1 Stabilizes Noncanonical Chromatin Structures

HMO1 stabilizes repeat tracts. On rDNA and on ribosomal protein gene promoters, HMO1 appears to exert an effect in large part through its association with nucleosome-free DNA or DNA associated with fragile nucleosomes. The potential instability of DNA containing repetitive sequence elements, such as that characterizing the rDNA array,

necessitates protective measures. In humans, long CAG repeat tracts underlie hereditary neurodegenerative diseases, including Huntington disease, as they have a propensity to expand. The length of CAG repeat tracts correlates with their instability; duplex DNA exhibits unusual flexibility, and unwound DNA may engage in intramolecular base pairing to form hairpin structures that hinder DNA replication (192). When embedded in the yeast chromosome, CAG repeat tracts are bound and stabilized by HMO1, which establishes a noncanonical chromatin organization (193). The length of CAG repeat tract chromatin that is protected from nuclease digestion is shorter than that protected by a nucleosome, raising the possibility that tetramer cores of histones associate with the DNA and that HMO1 may have replaced H2A and H2B, perhaps serving as a linker between tetramer cores.

Replication fork encounters with transcription. Recombination events and genomic instability may also be triggered by clashes between replication and transcription (194). Dedicated topoisomerases such as Top2p relieve the topological constraints that result when a replication fork encounters transcription and promote fork progression. In S phase, intergenic regions close to some transcribed genes exhibit low nucleosome density but accumulate both HMO1 and Top2p; together, Top2p and HMO1 appear to suppress chromosome fragility at the M/G_1 transition (195). Top2p binding occurs independently of HMO1, while a function of HMO1 may be to maintain the low nucleosome density required to facilitate Top2p-mediated DNA looping, which promotes the formation of topological domains and gene transcription.

DNA damage response. DNA damage may lead to events ranging from mutagenesis to chromosomal rearrangements. The DNA damage response (DDR) allows for a delay of cell cycle progression to ensure DNA repair and replication of the genome by high-fidelity polymerases and to mediate fork restart (196). The error-free mode involves a recombination event in which the newly synthesized strand is used as the template for replication of the damaged strand, whereas the error-prone mode relies on *trans*-lesion synthesis. This pathway choice is important for genome integrity. Among myriad events associated with replication, DNA topological changes include the sister chromatid bridges that form when replication forks pass through transcriptionally active chromatin loops (195). The association of HMO1 with such junctions has also been implicated as one of the mechanisms by which HMO1 promotes the error-free DNA damage tolerance pathway by facilitating template switching (197), and it is consistent with the preferred binding of HMO1 to four-way DNA junctions compared to linear DNA (132). These functions of HMO1 require its C-terminal extension (197), shown to be required for DNA bending and bridging (133, 134).

HMO1 and DNA Double-Strand Break Repair

HMO1 stabilizes chromatin. The absence of HMO1 does not affect the bead-and-string pattern of nucleosomes but makes the chromatin hypersensitive to nuclease (128, 198), suggesting that HMO1 stabilizes chromatin. A genome-wide analysis of HMO1 binding revealed extensive yet variable associations across the genome, with particular enrichment at genes encoding ribosomal proteins and rRNA (149). The coverage of HMO1 binding would be consistent with an effect on chromatin stabilization that is detectable by analysis of bulk chromatin.

DSBs are induced in the context of chromatin. The presence of sister chromatids allows repair by homologous recombination, whereas nonhomologous end joining operates without involving a separate copy of the DNA duplex. A number of histone modification and chromatin-remodeling events precede DNA repair pathway choice and render the chromatin template accessible to repair proteins (199). In yeast, a DSB may be site-specifically created at the mating-type locus *MAT* by inducing the expression of HO endonuclease. HMO1 binds this locus, and events involved in DSB repair include the eviction of HMO1 proximal to the DSB site (177). Notably, events such as histone H2A phosphorylation, INO80 recruitment, and nucleosome disruption (as evidenced by H3 eviction) occur faster in the absence of HMO1, suggesting that the absence of HMO1 creates a more accessible chromatin state. The facilitated chromatin remodeling is associ-

ated with more efficient DNA end resection, recruitment of DNA repair proteins, and repair by both homologous recombination and nonhomologous end joining. Consistent with the roles of HMO1 in maintaining nucleosome-free DNA segments, restoration of the original chromatin state also occurs faster in the absence of HMO1 (177).

Replication-independent endogenous DNA DSBs occur spontaneously and are not pathological lesions in that they do not induce mutation or cell death. Instead, they may possess important biological functions, perhaps in relieving topological stress that might otherwise result in uncontrolled DNA breakage. They have also been reported to occur nonrandomly, for example, with an increased frequency in heterochromatin (200). Such breaks are repaired by either Ku- or Rad51p-dependent pathways, as evidenced by the increased levels of such breaks in cells deleted for either Ku or Rad51p (201). Conversely, deletion of HMO1 resulted in reduced break levels, an outcome that would be consistent with the absence of HMO1 facilitating chromatin-remodeling events required for their elimination.

The HMO1 C-terminal domain is required for chromatin stabilization. The finding that chromatin-remodeling events associated with DSB repair occur faster in the absence of HMO1 points to a role for HMO1 in stabilizing chromatin. Notably, this stabilization requires the lysine-rich C-terminal domain, which was previously shown to be required for bridging separate DNA strands (134, 177). A role for the basic extension in chromatin stabilization is corroborated by the increased sensitivity to MNase characteristic of chromatin isolated from both *hmo1*Δ cells and cells expressing HMO1 deleted for its C-terminal extension (198). Notably, the expression of human histone H1 in *hmo1*Δ cells restores wild-type levels of MNase sensitivity, and it also complements the *HMO1* deletion in terms of the faster chromatin-remodeling events characteristic of *hmo1*Δ cells (198). HMO1 deleted for its lysine-rich CTD still binds chromatin and can compete with H1 for binding. Taken together, these observations suggest that HMO1 can fulfill the role of a linker histone and that it shares with mammalian H1 the requirement for a lysine-rich domain for chromatin stabilization to be manifest.

While the exact nature of the HMO1 interaction with nucleosomes remains unknown, the preferred binding of HMO1 to four-way DNA junctions is consistent with binding at the nucleosome dyad, as reported for H1, which likewise binds preferentially to DNA junctions (Fig. 2) (38). Based on the inference that DNA bending and underwinding by mammalian HMGB may facilitate nucleosome unwrapping, HMO1 might likewise be expected to destabilize nucleosomes; however, the observation that interactions between the HMO1 box A domain and the CTD are required for DNA bending offers an alternative scenario (118, 133, 134). It is conceivable that the HMO1 lysine-rich domain contacts DNA directly when HMO1 associates with nucleosomes, a circumstance in which DNA bending by HMO1 would likely be attenuated (Fig. 6), thus allowing HMO1 to stabilize the nucleosome. Thus, we propose a model in which HMO1 binds near the nucleosome dyad axis, most likely with its box A domain, which has the greatest preference for four-way junction DNA; such an interaction might free the lysine-rich CTD to interact with linker DNA, preventing DNA bending and generating a compacted nucleosome array with limited MNase access.

Interplay between Hho1p and HMO1

Both genes encoding core histones and the *HHO1* gene are transcribed in S phase, suggesting that Hho1p acts in concert with the core histones (202). Consistent with this observation, Hho1p binds the DNA entry/exit points of nucleosomes (56). However, during vegetative growth, the absence of Hho1p does not affect global chromatin structure, as evidenced by changes in MNase sensitivity (56, 198). A contributing factor may be its relatively low abundance, estimated to be 1 Hho1p molecule per 37 nucleosomes, a cellular content that is ~10-fold lower than that of HMO1 (57, 58, 60). Furthermore, inactivation of *HHO1* does not result in growth or mating defects, significant global changes in gene expression, or a change in average nucleosome distance (57). Phenotypes associated with *HHO1* deletion are subtle and have pointed to roles for Hho1p in suppressing homologous recombination and the formation of

silent chromatin (perhaps by affecting the function of the Sir complex), in promoting the formation of chromatin loops, and in chromatin compaction during stationary phase (59, 62–64). Disruption of *HHO1* results in a substantial increase in the levels of its own transcript, suggesting a feedback system for *HHO1* gene regulation (57). Curiously, a feedback mechanism for the regulation of the *HMO1* promoter was also suggested by the increased *HMO1* promoter activity in an *HMO1*Δ strain (135).

Another commonality between Hho1p and HMO1 is their preferred binding to rDNA. For Hho1p, this localization to rDNA is associated with a repression of recombination and with efficient transcriptional silencing by the compaction of rDNA chromatin (61, 85), whereas functions of HMO1 range from rDNA compaction during starvation to participating as a component of the Pol I transcription machinery, as discussed above (149, 150, 152, 154–156). While the inactivation of *HHO1* causes a modest enrichment of HMO1 at rDNA, the absence of HMO1 results in a significant increase in the association of Hho1p (198). The finding that the absence of either HMO1 or Hho1p results in a reciprocal increase in the binding of the other protein at rDNA may explain why the elimination of both proteins is required to render rDNA significantly more susceptible to MNase digestion. This interdependence appears to be specific to rDNA; for example, both HMO1 and Hho1p bind the mating-type locus *MAT*, yet the absence of one protein does not affect the binding of the other, and only HMO1 can protect linker DNA from MNase digestion at this locus (198).

CONCLUSIONS

The evolutionarily variable linker histones are critical for the stabilization of nucleosomes by binding DNA entering and exiting the core particle and by facilitating higher-order organization (15). The lysine-rich CTD of metazoan H1 is crucial for such stabilization; indeed, single-domain linker histones corresponding to the CTD are, for example, found in kinetoplastids, and even eubacteria have been shown to compact their genomic DNA by means of proteins with low-complexity lysine-rich extensions (45, 203). The lysine-rich C-terminal domain of HMO1 is unique for HMGB proteins and in marked contrast to mammalian HMGB proteins (and even yeast HMO2) that have acidic tails. The absence of HMO1 or deletion of the HMO1 CTD makes chromatin hypersensitive to nuclease, and it facilitates chromatin-remodeling events associated with DSB repair phenotypes that are complemented by the expression of human H1 in the *hmo1*Δ strain, pointing to a role for HMO1 in chromatin stabilization. Establishing the nature of the HMO1 interaction with reconstituted nucleosomes will be important to shed further light on the mechanism by which HMO1 executes such H1-like nucleosome stabilization. However, the ability of HMO1 to stabilize genomic DNA appears to go beyond conventional linker histone function. HMO1 plays a role in transcription by both RNA Pol I and Pol II, and it functions in the DNA damage response by directing lesions toward the error-free pathway. In these circumstances, HMO1 is required for the stabilization of nucleosome-free DNA or DNA associated with fragile nucleosomes. Taken together, emerging data suggest that yeast linker histone function is a division of labor between Hho1p and HMO1 in which Hho1p may have acquired more specialized functions due to its unusual domain organization, whereas the terminal lysine-rich extension of HMO1 has endowed it with the ability to stabilize both noncanonical and conventional nucleosome arrays.

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