H1 and HMGB1: modulators of chromatin structure

Jean O. Thomas1 and Katherine Stott
Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Cambridge CB2 1GA, U.K.

Abstract
Histone H1 and HMGB1 (high-mobility group protein B1) are the most abundant chromosomal proteins apart from the core histones (on average, one copy per nucleosome and per ten nucleosomes respectively). They are both highly mobile in the cell nucleus, with high on/off rates for binding. In vivo and in vitro evidence shows that both are able to organize chromatin structure, with H1 binding resulting in a more stable structure and HMGB1 binding in a less stable structure. The binding sites for H1 and HMGB1 in chromatin are partially overlapping, and replacement of H1 by HMGB1 through the highly dynamic nature of their binding, possibly facilitated by interaction between them, could result in switching of chromatin states. Binding of HMGB1 to DNA or chromatin is regulated by its long and highly acidic tail, which is also involved in H1 binding. The present article focuses mainly on HMGB1 and its interaction with chromatin and H1, as well as its chaperone role in the binding of certain transcription factors (e.g. p53) to their cognate DNA.

Introduction
Histone H1 and HMGB1 (high-mobility group protein B1) are considered together in the present paper because these two structurally unrelated proteins, whose modes of binding to chromatin are completely different, may be functionally linked and act in opposition with respect to the stability of chromatin structure. The linker histone (H1) family of proteins are intrinsic chromatin proteins and bind with a stoichiometry of approximately one copy per nucleosome on average [1]; they are approximately 10 times more abundant than HMGB1 and HMGB2, much less mobile [2] and bind to chromatin with higher affinity. H1 has a central folded globular (G) domain, which binds at the nucleosome dyad [3] and stabilizes nucleosome structure, flanked by a basic N-terminal tail and a much longer, highly basic, C-terminal tail, which organizes and neutralizes internucleosomal linker DNA [4,5]. H1 is required for the formation of stable well-ordered higher-order chromatin structure [6]. In contrast with H1, HMGB1 and the closely related HMGB2 have been implicated in a variety of DNA transactions in which chromatin structure might need to be loosened in some way and/or in which the DNA is distorted. These include transcription, replication, recombination and DNA repair [7–9]. In the present paper, we focus mainly on HMGB1 and its properties and interactions in the nucleus of higher eukaryotes. The recent explosion of interest in HMGB1 as a non-nuclear/secrected pro-inflammatory cytokine, with RAGE (receptor for advanced glycation end-products) among its targets (reviewed in [10]), will not be discussed.

A distinguishing property of HMGB1 in the nucleus is sequence-independent DNA binding and bending, and its ability to bind preferentially to pre-bent or distorted DNA (reviewed in [8,9]). DNA binding is a property of its two basic homologous HMG (high-mobility group)-box domains (A and B), which are linked by a short basic linker. DNA bending, towards the major groove, occurs as a result of minor-groove bending and intercalation into DNA of bulky amino acid side chains on the DNA-binding faces of the two HMG boxes. The long, homogeneously acidic, C-terminal tail (30 residues in HMGB1, 20 in HMGB2) which is linked to the boxes by a basic linker, performs a regulatory function and lowers the DNA binding affinity of the boxes [11,12]. The tail is required for preferential binding to pre-bent (e.g. minicircle) DNA over linear DNA in an in vitro competition assay [13], as also shown for the single HMG box protein HMG-D [14], and is necessary for transcriptional activation in a transfection assay [15,16]. The acidic tail also turns out to play a role in binding of HMGB1 to chromatin through interaction with core and linker histones, as discussed below. HMGB1, through DNA bending, may have a chaperone role in facilitating the binding to DNA of other proteins; it interacts with a number of (unrelated) DNA-binding proteins, and this may in some cases serve to recruit HMGB1 to particular chromatin sites (see below).

The binding sites for H1 and HMGB1 in chromatin may partially overlap, and they can both contribute to chromatin structural organization. HMGB1, like H1, binds to linker DNA [17] close to the nucleosome dyad where the linker DNA enters and exits; H1 also interacts with the nucleosome core DNA through its central globular domain. Moreover, both H1 and the Drosophila single-HMG-box protein HMG-D increase the nucleosome repeat length of chromatin in an in vitro system [18]. However, H1 supports a more stable chromatin structure, and HMGB1 and its counterparts support a looser one. This is shown clearly in vivo, in both Drosophila [19] and Xenopus [20] early embryos, where there is no H1 before the mid-blastula stage, but there is abundant HMGB-box protein (HMG-D or B4 respectively).

Key words: high-mobility group box (HMG box), high-mobility group protein B1 (HMGB1), histone H1, histone H3, NMR spectroscopy, p53.
Abbreviations used: HMG, high-mobility group; HMGB1, high-mobility group protein B1; HSQC, heteronuclear single-quantum coherence; PRE, paramagnetic relaxation enhancement; SAXS, small-angle X-ray scattering; TAD, transactivation domain.
1To whom correspondence should be addressed (email jot1@cam.ac.uk).
Figure 1 | Intramolecular association of the HMGB1 acidic tail with the HMG boxes  
(A) NMR chemical-shift changes (expansions of selected resonances shown on the right) in the $^{15}$N-HSQC spectrum of HMGB1 due to successive five-residue truncations show the path of the acidic tail. Reproduced from [24] with permission.  
(B) Schematic diagram of a dynamic compact structure for HMGB1, which is supported by additional evidence (SAXS, PRE). Reproduced from [25] with permission.

The chromosomes of the rapidly dividing embryos are well organized and fully functional, but less compact than they are at later developmental stages when the HMGB1 has been replaced through successive cell divisions by accumulating H1. In this sense, H1 and HMGB1 act in opposition in dynamic chromatin transitions [21].

Molecular basis of the regulatory role of the HMGB1 acidic tail

The acidic tail reduces the affinity of the HMG boxes of HMGB1 and HMGB2 for linear DNA significantly [11,12], but the effect is much less marked for binding to DNA mini-circles, in which the pre-bent DNA is a preferred substrate for HMGB1 binding [13]. There are quantitative differences in the DNA-binding and -bending properties of HMGB1 and HMGB2, all consistent with the stronger binding of HMGB2 [13]. The differences are due to different acidic tail lengths: 30 aspartate and glutamate residues in the case of HMGB1 and 20 for HMGB2. When the tails are removed, the properties of the tandem HMG boxes of HMGB1 and HMGB2 are essentially identical, and when the HMGB1 tail is replaced by the shorter tail of HMGB2, the hybrid molecule has the DNA-binding and -bending properties of HMGB2 [13].

In principle, the tail might modulate (decrease) DNA binding by the HMG boxes simply through non-specific charge repulsion of the DNA by the negatively charged tail. Alternatively, the tail might interact with the DNA-binding regions on the HMG boxes. The protein has resisted attempts at crystallization, and the lack of sequence diversity in the acidic tail (hence overlap of resonances) precludes NMR structure determination of the whole protein. However, following up earlier indications of tail-box interaction ([22,23], and K.B. Lee and J.O. Thomas, unpublished work), we gained detailed insights through a systematic ‘tail-truncation’ approach in conjunction with NMR chemical-shift mapping (Figure 1A). A series of mutants with sequential five-residue deletions was examined [24], and successive chemical-shift changes in the box residues revealed that (apart from the last five residues) the tail followed a relatively...
well-defined path on the surface of the HMG boxes and interacted with the two basic linkers. The distal five residues of the tail appeared to interact promiscuously and non-specifically with the surfaces of the two boxes, and would, in principle, be more readily available than the rest of the tail for interaction with other partners. The mapping studies revealed unambiguously that the DNA-binding faces of the two HMG boxes (helices I and II) were occluded by the acidic tail, thus clearly revealing the basis of the regulatory role of the tail in DNA binding. The salt-dependence of the chemical-shift perturbations revealed that the A box and the linkers were less tightly bound to the tail than the B box, which remained largely bound in 200 mM NaCl [25].

The consequence of the extensive interaction of the acidic tail with the boxes and linkers is compaction of the tertiary structure: the tail is sandwiched between the boxes (and linkers) and draws them closer together (Figure 1B). Support for such a ‘collapsed’ structure comes from 15N-relaxation NMR, SAXS (small-angle X-ray scattering) and PRE (paramagnetic relaxation enhancement) measurements [25]. The NMR measurements (R2/R1) show that the tail causes the two domains, which, in absence of the tail, tumble independently (behaving as a protein of molecular mass of approximately 10 kDa, rather than the actual ~20 kDa) to become ‘less dynamic’ and tumble together as a whole, as expected of a protein of molecular mass of approximately 30 kDa. The shape of the X-ray scattering profile for the full-length protein is no longer compatible with a dumb-bell-shaped molecule characteristic of the ‘tail-less’ protein. The maximum radii for the full-length and tail-less protein are, however, very similar, and computational modelling of the SAXS data shows that the best fit for the full-length protein is with a dynamic assembly of compact and extended forms (Figure 1B). The proximity of the two HMG boxes in the compact form is shown clearly by PRE measurements; when a paramagnetic tag is attached to Cys44 on the DNA-binding face of the A box, NMR resonances on the DNA binding face of the B box, specifically helices I and II, are reduced in intensity (due to interaction of the unpaired electron of the nitroxide group in the tag with the protons in the protein). Moreover, the PRE data suggest that there is dynamic averaging of the ‘bleaching’ of the signal over the two helices, again suggesting a mobile assembly, rather than a single, unique, conformation.

**HMGB1 and chromatin**

Since HMGB1 and H1 bind in the same general location (near to/at the nucleosomal DNA exit/entry sites), their binding sites are likely to overlap, and binding of H1 and HMGB1 may be mutually exclusive [26]. This could account for a population of mononucleosomes preferentially released upon micrococcal nuclease digestion of mouse myeloma nuclei that contain stoichiometric amounts of HMGB1, but no H1 [27]. Windows of opportunity for replacement of one protein by the other in chromatin could arise due to the high on/off rates of association of H1 and HMGB1 with chromatin, shown by FRAP (fluorescence recovery after polarization) measurements on GFP (green fluorescent protein)-tagged proteins transfected into cultured mammalian cells [2]. For example, the particularly mobile HMGB1 could effectively displace H1 by competing for its binding site, or part of it, resulting in local destabilization of chromatin and possibly recruitment of other proteins, leading to transcriptional activation; a nucleosome-specific instance of H1 replacement by HMGB1 has been described [28]. Competition between H1 and HMGB-D for binding sites in bulk chromatin has also been demonstrated [18].

The location of HMGB1 on the linker DNA, close to the nucleosome core, places it in close proximity to the H3 dimer component of the histone octamer, whose N-terminal tails interact with linker DNA [29] and are likely to be competed off when HMGB1 binds. HMGB1 has been reported to interact through its acidic tail with the flexible N-terminal tail of H3 in a mixture of core histones, and this interaction appears to be required for transcriptional enhancement by HMGB1 in a transfection assay [16], the last five residues of HMGB1 (DDDDE) being reported to be essential for the interaction. We have confirmed the H3–HMGB1 interaction in chromatin by cross-linking and other methods, but using a set of HMGB1 truncation mutants, we find that, in a chromatin context, the length of the HMGB1 acidic tail is irrelevant, provided at least five acidic residues remain (M. Watson, H. Fischl, K. Stott and J.O. Thomas, unpublished work). However, we also confirmed that, for free HMGB1 and a histone H3 N-terminal peptide, the last five acidic tail residues were indeed important for interaction; this is presumably a consequence of the intramolecular shielding of the acidic tail in the free protein, due to its binding to the HMG boxes, leaving only the last five residues unbound [24] and available for interaction with H3. However, in the presence of DNA the length of the acidic tail, beyond a minimum length of five residues, was again irrelevant for the interaction of the free proteins – a consequence of competition by DNA for the HMG boxes and release of the acidic tail for interaction with H3. NMR studies of HMGB1 and a 15N-labelled H3 N-terminal peptide (residues 1–40) showed that regions on the H3 peptide that interact most strongly with HMGB1 are predicted to have helical propensity; there are also weaker interactions elsewhere (M. Watson, H. Fischl, K. Stott and J.O. Thomas, unpublished work). Further studies will show whether these regions, which are essentially unstructured in the free peptide, do adopt helical character upon interaction.

The structural consequence of HMGB1 binding at linker DNA, and bending of the DNA, is to introduce a distortion that would be transmitted to the body of the nucleosome [21], destabilizing it. When HMG-D is bound, the nucleosomal DNA at the dyad and one terminus (which are close together) become more nuclease-sensitive, reflecting local changes in accessibility [30]. The acidic tail [which is short (12 residues) in the case of HMG-D] is necessary for this effect, possibly because it stabilizes the DNA distortion, by interacting electrostatically with the basic regions of the
histones that are exposed as local histone–DNA interactions are disrupted [21]. These regions might be in the structured histone core, but might also be in the H3 tail, which is likely to have been displaced from the linker by the binding of the HMG box. Consistent with this destabilizing mode of action, HMGB1 facilitates histone octamer migration mediated by the ACF (ATP-utilizing chromatin assembly and remodelling factor)/CHRAC (chromatin accessibility complex) remodelling complex, for which the HMGB1 acidic tail is essential [31], and several remodelling complexes include an HMG-box subunit as part of the intrinsic machinery (e.g. BAF57 in human SWI/SNF and SSRP1 in FACT (reviewed in [8])).

Interaction of HMGB1 with linker histones

The mobility in the nucleus of H1 and the even higher mobility of HMGB1 would, in principle, provide a means for replacement of H1 by HMGB1, as discussed above. H1 binds to DNA and chromatin more tightly than HMGB1, and prior modification (e.g. by phosphorylation, or by poly-ADP ribosylation [28]) to lower its affinity might be necessary. The replacement process might also be facilitated by direct interaction between H1 and HMGB1; such an interaction has been demonstrated in vitro and is stable at ‘physiological’ ionic strength [26] (Figure 2). The interaction is progressively lost as the acidic HMGB1 tail is successively truncated by up to 20 residues, indicating a role for a substantial part of the tail in binding to some part of H1. The acidic tail is normally associated for much of the time with the HMG boxes as described above, and NMR spectroscopy shows clearly that H1 competes with the boxes for the HMGB1 tail; the chemical shifts arising from the HMG boxes in 15N-HSQC (heteronuclear single-quantum coherence) spectra are perturbed by interaction with H1, and shift to the same extent as when the acidic tail is truncated by 20 residues (Figure 2A). H1 thus lifts the HMGB1 acidic tail off the HMG boxes. In the reciprocal experiment, NMR spectroscopy has identified the region of H1 involved in the interaction as its basic C-terminal tail; although the H1 tail resonances are heavily overlapping in a crowded region of the 15N-HSQC spectrum, it is clear that many of them shift upon interaction with HMGB1, whereas the well-dispersed globular domain resonances do not [26].

The likely consequences of an interaction between the acidic C-terminal tail of HMGB1 and the basic C-terminal

---

**Figure 2** Interaction between H1 and HMGB1 through their C-terminal basic and acidic tails respectively

(A) NMR chemical-shift mapping of the interaction of [15N]HMGB1 with H1. (B) Interaction of H1 and HMGB1 (1:1 molar ratio) shown by a time course of chemical cross-linking with two different cross-linking reagents, DMS (dimethyl suberimidate) and EDC [1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide], and analysis in an SDS/18% polyacrylamide gel. (C) Schematic showing that H1 pulls the acidic tail (green) off the HMG boxes (red and blue), as revealed by the chemical-shift mapping shown in (A), and that the interaction is between the acidic C-terminal tail of HMGB1 and the long basic C-terminal tail of H1. Adapted from [26] with permission.
tail of H1 are obvious. The affinity of HMGB1 for DNA and chromatin will be increased (because the DNA-binding faces of the boxes are no longer shielded), and the affinity of H1 will be decreased (because the positive charge on the long basic C-terminal tail, which organizes the exiting and entering linker DNA [4,5], as well as providing counterions for the DNA phosphate groups, will now be at least partly shielded by the HMGB1 acidic tail). Since the two proteins are highly mobile, such interactions might occur in solution and lead to replacement of resident H1 by ‘visiting’ HMGB1. Or they might occur as a result of transient release of the C-terminal domain of chromatin-bound H1 in chromatin, without loss of the entire molecule. Additional studies are necessary to probe this further.

**Chaperone role for HMGB1**

HMGB1 also has another slightly different role in the cell nucleus, essentially acting as a chaperone that facilitates binding of various transcription factors [including Oct-1 and Oct-2, p53, Hox proteins, steroid hormone receptors and TBP (TATA-box-binding protein)] to their cognate DNA-binding site [8,9]. The transient nature of its binding [2] makes HMGB1 an excellent candidate for a general factor that enables the efficient formation of various short-lived interactions in nucleoprotein complexes. We have studied p53 in some detail in order to gain insights into the nature of the chaperone role of HMGB1 in p53 binding.

HMGB1 stimulates p53 DNA binding to linear DNA in vitro and increases p53 activity in vivo, in a transfection assay [32]. That binding to pre-bent (e.g. minicircle) DNA is not facilitated by HMGB1 [33] suggests that HMGB1 promotes binding to linear DNA through its DNA-bending activity. It is known that the B box is by far the more effective at bending linear DNA (presumably due to two intercalating residues on its DNA-binding face), whereas the A box (which has only one) binds preferentially to pre-bent DNA [34–36].

There is no evidence for a ternary complex containing linear DNA, p53 and HMGB1 in gel-shift assays (although such a complex has been reported for hemi-catenated DNA [37]). The individual HMG boxes, A and B, can also facilitate binding [33]. Direct interaction of p53 and HMGB1 has been shown by Western blotting [32] and immunoprecipitation [38]; phage display showed PXXPXP to be one of the preferred HMGB1-binding sequences, leading to the suggestion that HMGB1 interacts with p53 through the PXXPXP motif in its intrinsically disordered N-terminal proline-rich region (residues 64–92) [39]. The similarly disordered TAD (transactivation domain) (residues 1–63) and another disordered region of similar size at the C-terminus (comprising the tetramerization and regulatory domains of p53), which flank the folded DNA-binding domain, are heavily post-translationally modified in vivo and provide binding sites for a variety of partners [40].

We have shown using chemical cross-linking and biophysical measurements that the isolated A and B boxes of HMGB1 both interact with the TAD, but not with the PXXPXP region. Full-length HMGB1 interacts only weakly with p53, whereas the tail-less protein interacts much more strongly, presumably because the acidic tail shields the boxes as discussed above. We have studied in detail the interaction of the p53 TAD with the HMG boxes of HMGB1, and have identified the interacting regions on each partner (J.P. Rowell, K.L. Taylor, K. Stott, M. Watson and J.O. Thomas, unpublished work). Although we found no binding of the p53 TAD to full-length HMGB1 in our assays, it is possible that free p53 and HMGB1 do interact in vivo, before chromatin binding, because of the dynamic nature of the binding of the tail to the HMG boxes discussed above. Such interaction would then provide a means of recruiting HMGB1 to the appropriate location in chromatin. The acidic tail would probably not be a hindrance to the interaction of ‘visiting’ HMGB1 with bound p53 (we suggest through the A box), since the tail would be displaced from the boxes upon binding of HMGB1 to linker DNA (we suggest through the B-box) and would presumably be captured by H3 (see above), or another protein, or possibly some other part of p53 itself. At least some p53-responsive promoters (e.g. the p21 promoter [41]) are indeed assembled into chromatin (rather than being located in a nucleosome-free region), so H3 could well be the acceptor for the acidic tail.

**Concluding remarks**

Both H1 and HMGB1 consist of DNA-binding domain(s) and charged disordered tail regions. The negatively charged tail of HMGB1 sequesters its own DNA-binding boxes and linkers; it may be displaced from them by a variety of basic components of chromatin, including H1. In both H1 and HMGB1, the tails modulate the binding affinity of their structured domains. However, a key aspect of the function of the tails lies in their capacity to facilitate change through an ability to bind and switch between different inter- and intra-molecular substrates.

**Funding**

Work on HMGB1 and H1 in J.O.T.’s laboratory was supported by the Biotechnology and Biological Sciences Research Council [grant number BB/D002257/1] and by the Medical Research Council [grant number G0401547], awarded through the European Science Foundation (ESF) European Collaborative Research (EUROCORES) EuroDyna Programme.

**References**


12 Stros, M., Stokrova, J. and Thomas, J.O. (1994) DNA looping by the HMGB-box domains of HMGB1 and modulation of DNA binding by the acidic C-terminal domain. Nucleic Acids Res. **22**, 1044–1051


