Minireview

The changing paradigm: estrogen receptor α recognition on DNA and within the dynamic nature of nucleosomes

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Abstract: Estrogen receptor alpha (ERα) plays a major role in the expression of estrogen-responsive genes. Although its conventional binding characteristics have been considered coincident with & exclusively in the class of steroid hormone receptors, increasing evidence challenges this paradigm. ERα was shown to bind to consensus estrogen response element half-sites (cHERE) in DNA in the presence of the ubiquitous, abundant & conserved architectural protein, high mobility group protein 1 (HMGB1). It also binds to direct repeats with various spacers, in addition to everted repeats. These *in vitro* binding sites have been shown to be active *in vivo*, with both the binding affinity and transcriptional activity increased in the presence of HMGB1. Surprisingly, ERα does not bind to the optimally oriented cERE at the dyad in rotationally phased and translationally positioned nucleosomes. However, the presence of HMGB1 restructures the nucleosome to facilitate increased ERα accessibility, resulting in sequence-specific estrogen receptor binding. The finding that HMGB1 interacts with unbound ERα provides a unique avenue for enhanced ERα activity and possibly an increase in the extent of targeting at estrogen-responsive genes. The findings are consistent with ERα 1) targeting a much wider selection of genomic response elements (half-sites and inverted, direct and everted repeats) and 2) exhibiting characteristics of both steroid and non steroid nuclear receptors. Growing evidence already shows a competition occurs at the DNA level between ERα and the non steroid nuclear hormone receptor, thyroid receptor (TR). Collectively, these reports suggest a less restrictive cataloging for estrogen receptor and a broader paradigm for understanding its role in the regulation of estrogen-responsive genes and influence on non steroid hormone receptor activities.

Keywords: estrogen receptor; HMGB1 protein; nucleosome restructuring; conformational selection

1. Introduction

Steroid hormone receptors are members of a superfamily of nuclear hormone receptors (NHRs; orphan receptors are not discussed) that are ligand-activated transcription factors. This family also includes the thyroid receptors, vitamin D receptors and two categories of retinoic acid receptors (RAR and RXR). The NHRs have a common modular structure, which includes an N-terminus transcriptional activation domain, the DNA binding domain (DBD) and a hinge region that connects to the carboxyl-terminal ligand-binding domain (LBD). Since they activate transcription by binding to their response element in DNA, they have been conventionally cataloged according to whether their hormone response element (HRE) on DNA is an inverted repeat (IR) or a direct repeat (DR) of half-sites, the number of bases (n) in the spacer between half-sites and whether the hormone receptor binds the HRE as a homo-or hetero-dimer (Table 1) [1]. In considering only Class I and II in this Minireview and Hypothesis, it is interesting that, with the exception of the estrogen receptor, all the steroid hormone receptors recognize the same six base half-site consensus sequence. 5'-AGAACA-3' (cHRE) in DNA, while ER recognizes a different consensus half-site sequence, 5'-AGGTCA-3' (cHERE), which is also recognized by the non steroid hormone receptors.

Table 1. Nuclear hormone receptors.

Steroid (Class I)	Non-Steroid (Class II)
Estrogen, ER	Thyroid, TR
Androgen, AR	Retinoid, RXR
Progesterone, PR	Retinoic Acid, RAR
Mineralocorticoid, MR	
Half-sites	
5'-AGGTCA-3' (ER)	5'-AGGTCA-3' (all)
5'-AGAACA-3' (all others)	
Binding	
Inverted Repeats ($n = 3$bps)	Direct Repeats ($n = 3$–5)
Homodimers	Predominantly Heterodimers

Estrogen has multifaceted roles of activating a regiment of genes that impinge on a multitude of human physiological processes, including differentiation, reproduction and metabolism. Along with this, estrogen has been implicated in the pathophysiology of numerous diseases [2].

Estrogen acts on the genomic level by mediating its effect through its interaction with estrogen receptors, of which ERα will be the focus in this review. ERα binds to its response element as a homodimer, with the DBD making contacts in the major grooves of the estrogen response element half-sites (cHERE) that are separated by a 3 bp spacer. However, although mounting data show that ERα also binds to a wide variety of cHERE arrangements and estrogen response units (ERU) that contain more than one ERE-like sequence [3,4,5], and are linked to regulated expression of a number of genes [3,6,7,8], the original classification of ERα as a Class I receptor has not been challenged.

The range of activities of ERα is associated not only by the nature of the EREs, but also with the differential recruitment of coregulator proteins to the ER/ERE site, which then communicates with the transcriptional machinery to effect the regulation of transcriptional activation and the physiological response [9-12]. The focus here is on the influence of the HMGB1 coactivator protein,

a highly conserved, ubiquitous and abundant architectural protein. It binds nonspecifically to DNA, induces large bends [13,14,15], produces DNA flexure [16] to facilitate nucleoprotein assembly and the binding and enhancement of transcriptional activity in HMGB1-sensitive genes [17-29]. It has been further demonstrated that HMGB1 stimulates the translocation of the histone octamer on DNA by the chromatin remodeling complex, ACF/CHRAC [30]. HMGB1 has features similar to the FACT complex that contains an HMG box component and can remodel nucleosomes in an ATP-independent manner [31].

Defining the ERα binding characteristics to naked DNA provides insight into what may occur within the cell. However, within the nucleus, DNA is complexed with and supercoiled about the outside of an octamer of core histones to form a nucleosome, the fundamental repeating unit of the eukaryotic chromosome. The nucleosome adds an additional level of complexity since its intrinsic structure inhibits transcription factor access to the response elements and restricts gene expression. Genomic studies have shown that transcriptional activation by ERα is a multistep process and involves an ordered & cyclic recruitment of factors [32]. A model for ERα-mediated transcription in breast cancer cells proposes that a pioneer protein, such as FOX A, is important to target response elements and initiate the subsequent recruitment of necessary factors for transcription [33,34]. Finally, if the response element is within a nucleosome, chromatin remodeling complexes (CRCs) may cooperate to facilitate transcriptional activation by at least two means. These complexes can use either an enzymatic function to1) post transcriptionally modify the core histones to destabilize the nucleosome or 2) remodel the nucleosome by an ATPase activity and/or translate the histone octamer to a new position so that the response element in DNA is more accessible to a regulatory factor, such as ERα [35,36,37].

2.   Current findings

2.1. ERα-DNA binding exhibits plasticity and is enhanced by HMGB1 protein

Electrophoretic mobility shift assays (EMSA) showed that ERα binds strongly to cERE DNA and that changes in specific nucleotides in one or both of the half-sites reduces its binding affinity [3,8]. If all the nucleotides in one the half-sites are changed to drastically reduce the binding affinity, the Kd for ERα binding to the cHERE increases from ~ 2 nM in cERE to ~ 200 nM. However, in the presence of 400 nM HMGB1, ERα binds to this cHERE with an affinity of ~ 2 nM, a value essentially the same to what is observed for ERα binding to cERE in the absence of HMGB1 [4]. This led to investigating the extent to which ERα would bind to half-sites or multiple half-sites that were arranged as 1) palindromic sequences with different size spacers of n = 0, 1, 2, 3 & 4; 2) direct repeats, with spacers of 3, 15 & 20; 3) an everted repeat of spacer n = 6; and 4) an inverted repeat with a spacer of n = 24. Each of these constructs and the spacer sizes were derived from base pair changes in an original construct that contained two adjacent cEREs (Figure 1) [38].

Unexpectedly, even in the absence of HMGB1, the gel shift assays indicated ER (both ERα & ERβ) binds to all these cHERE arrangements to form a well-defined complex. In some cases, higher ERα levels drove the formation of a second ERα-DNA complex, which presumably contained two ERαs, each stabilized by interaction with only the half-site on DNA [38]. Therefore, even in the absence of HMGB1, ERα bound to multiple arrangements of half-sites (inverted, direct and everted

half-sites) that contained different numbers of bps in the spacer. In addition, the presence of HMGB1 produced a cooperative binding, with the second complex being the predominant species [38].
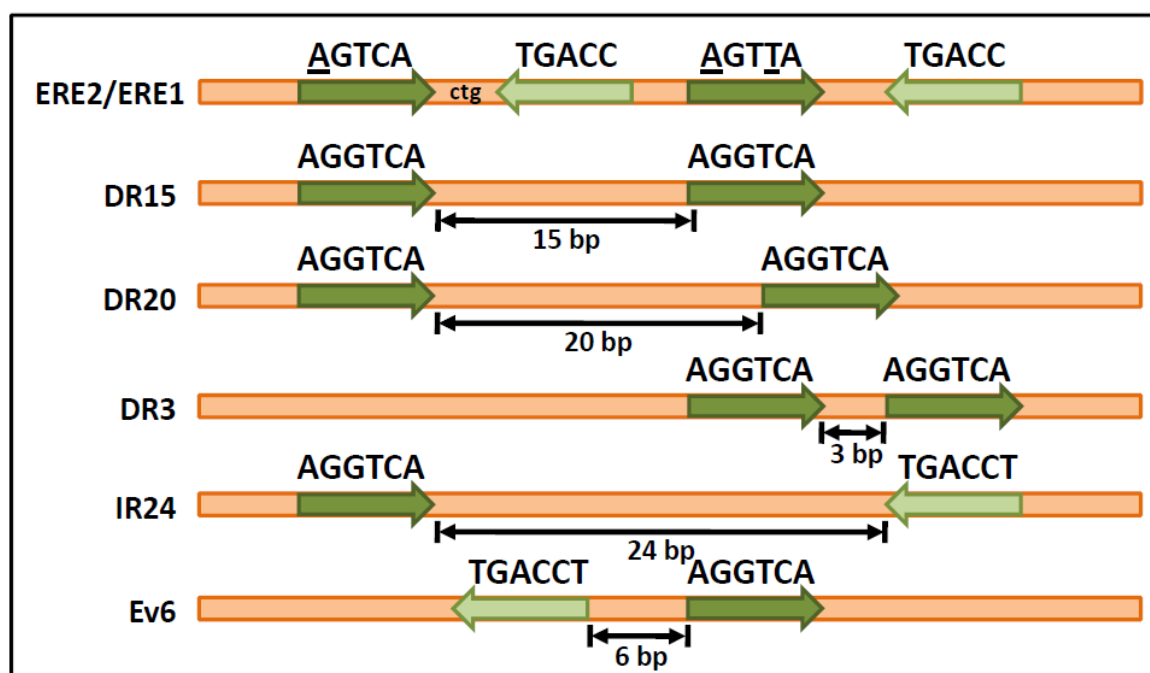

Figure 1. Schematic representation of ncEREs (DRs, EvR, and IR)[38]. The relative position and orientation of cHERE in the DNA constructs for DRs, an EvR (ER), and an IR. The spacing was derived from the positions of ERE2/ERE1 in the vitellogenin B1 ERU [4,63,64], with the original HEREs replaced with either cHERE or a sequence that ER does not bind to [4]. The number after the designated orientation (e.g. DR15) is the number of base pairs between the cHEREs.

2.2. Nonconventional ERE half-site arrangements drive luciferase expression in living cells and exogenous HMGB1 increases expression

Because the Kd value is an *in vitro* signature for binding affinity, it was important to determine if the binding affinity for nonconventional EREs (nEREs) would correlate with that observed in transcriptional activation assays within the cell. Therefore, an expression vector for ERα, along with a luciferase reporter construct that was driven by either 1) multiple tandem consensus EREs ($n = 1, 2$ & 3) or 2) multiple tandem half-sites (effectively direct repeats, cHEREn ($n = 1, 2$ & 3 bps); or 3) individual cEREs with a spacer of 0, 1, 2, 3 & 4 were transfected in the ERα-negative human osteosarcoma (U2OS) cell line. The level of luciferase expression showed that all the ERE target sites drove luciferase expression, with transcriptional levels increasing in a series, as there is an increasing number of cEREs or cHEREs in tandem [38]. In almost all cases, the transfection of the HMGB1 expression vector enhances the expression level [38].

To determine the extent to which HMGB1 stimulates gene expression, the expression of the endogenous HMGB1 gene in the U2OS cell line was knocked down (KD) by siRNA technology. The HMGB1 KD reduced the luciferase expression to less than 20% of control, with the HMGB1 protein

level reduced by 70% [38]. These results further demonstrated that cEREs, direct repeats of the cHERE and cEREs with alternative numbers of bps in the spacer can drive luciferase expression and that HMGB1 acts as an important coactivator and directly enhances estrogen-responsive gene expression.

2.3. Estrogen receptor does not bind to cERE within the canonical nucleosome

Since HMGB1 increased the binding affinity of ERα on DNA and enhanced transcription levels in the U2OS cell line, the possible influence of HMGB1 on 1) the nature of the nucleosome and 2) ER binding to nucleosomal DNA was examined. A 161 bp DNA was constructed with a cERE incorporated so that it would reside at the dyad within the nucleosome. Nucleosome positioning sequences were built into the DNA so that the cERE was "locked" into a rotationally phased and translationally positioned arrangement. This fixed the cERE so that the major grooves in the cERE, which bind ERα, were facing outward from the histone octamer and would be in the optimum orientation for strong ERα binding. The nucleosome was prepared by the salt dilution, histone exchange method [39]. The nucleosome (N) was purified by sucrose gradients, yielding a single band observed on EMSA, However, EMSA of the ERα binding affinity study revealed that ERα exhibited very little affinity for the nucleosomal DNA, with an estimated Kd ~ 300 nM [40]. One explanation for this might be that strong ERα/cERE binding within the context of a nucleosome requires more than the exposed major grooves of cERE, as would be accessible at the DNA level. In addition, the tails of the core histones may block access to the cERE and/or other ERα structural domains, which could be important at the nucleosome level. Alternately, ERα may require a "wrapping" around the DNA, which may be difficult since the histone octamer makes tight contacts with the DNA.

2.4. HMGB1 restructures the canonical nucleosome into two alternate states

Although ERα did not bind to the canonical nucleosome, preliminary experiments showed that the presence of 400 nM HMGB1 with the nucleosome appeared to enhance the ERα binding affinity. To define the nature of the nucleosome better, we incubated 1600 nM HMGB1 with the nucleosome and purified it by sucrose gradients. These nucleosomes occurred at the same elution volume as the canonical nucleosome. However, the EMSA revealed two bands, both that exhibited distinctly different mobilities than the canonical nucleosome, suggesting that HMGB1 had restructured the nucleosome to two alternate and stable forms, which we refer to as N′ and N″ [40].

Supershift experiments with antibodies to the core histones indicated that the histones were present in the restructured nucleosome. On the other hand, antibodies to HMGB1 failed to produce a supershift [40]. This suggested that HMGB1 bound transiently to the nucleosome and functions in a "hit-and-run" mechanism [4,41].

To determine if any HMGB1 was present with the nucleosome, a quantitative analysis of the HMGB1 level in the sucrose gradient fraction containing the restructured nucleosomes indicated that there was 25 nM HMBG1 [40], which corresponds to approximately 2 HMGB1 per nucleosome.

Importantly, the restructured nucleosomes were stable at − 20 °C for extended periods of time (months) and represent ideal systems to carry out extensive studies. Nucleosome challenge studies were done to determine their stability as a function of temperature, increasing [NaCl] and increasing levels of naked, unlabeled 161 bp DNA. At 37 °C, the EMSA showed that N′ and N″ became slowly unstable within a few hrs. Increasing [NaCl] gradually converted increasing amounts of N′ and N″

into the canonical nucleosome, N, with complete conversion observed at about 300 mM. Significantly, all three nucleosome forms were simultaneously present at intermediate levels of [NaCl], indicating an equilibrium mixture of the three forms. Strikingly similar to the effect of increasing [NaCl], increasing DNA levels also clearly showed the equilibrium mixture of the three forms, with the complete conversion of N′ and N″ forms into the canonical nucleosome form occurring at the higher levels of DNA [40]. We suggest that the nucleosome conversions that occurred from increasing [NaCl] were a result of NaCl weakening the electrostatic attraction between HMGB1 and the DNA/nucleosome, while increasing level of DNA served as a "DNA sink" to effectively compete away HMGB1 from interacting with the nucleosome [40]. In summary, these competition experiments show that the HMGB1-restructured nucleosomes (N′ & N″), which were in equilibrium with the canonical nucleosome, N, were converted to the N form by changing the solution conditions. In other words, the nature of the nucleosome is very sensitive to its immediate environment.

2.5. ERα binds to the HMGB1-restructured nucleosomal DNA

ERα binding profiles for the restructured nucleosomes indicated that the Kd value for ERα/cERE was 52 nM, about 6 times stronger than on the canonical nucleosome, yet still about 20 times weaker than on naked DNA. DNase I footprinting experiments showed that the ERα/cERE binding was sequence-specific [40]. This indicates that HMGB1 had restructured the nature of the nucleosome, in some manner, to facilitate ERα access to the cERE and exhibit strong and sequence-specific binding.

2.6. HMGB1 may disrupt the interaction of the core histone tails with DNA

An octamer of core histone proteins (H4, H3, H2A & H2B) make up the inner structure about which the DNA is superhelically coiled in a nucleosome. Although among the most evolutionarily conserved proteins, the core histones undergo post-transcriptional modifications, with most modifications occurring in the tails. The tails, which make up over 25% of the proteins, extend out from the DNA and are thought to interact with the DNA to modulate the function of the nucleosome, while not disrupting the core nucleosome structure, as evidence by the DNase I 10 bp pattern. [42].

To investigate the possibility that HMGB1 may disrupt the interaction of the positively charged histone tails with the DNA and thereby contribute to an increase in cERE accessibility, "tailless" nucleosomes (tlN) were prepared in the absence of HMGB1 [40]. The ER binding affinity on the tlN was compared with that of the canonical nucleosomes (N), in addition to determining the effect of HMGB1 on ERα binding. The EMSA results for the ERα reactions with the tailless nucleosomes (untreated with HMGB1) indicated the Kd was ~ 45 nM [40]. This ERα binding affinity is comparable to the Kd value observed for 1) nucleosomes in the presence of 400 nM HMGB1 and 2) the HMGB1-restructured nucleosomes, N′/N″, and is in marked contrast to the binding affinity to the canonical nucleosome. This shows that the presence of the core histone tails strongly influences ERα binding [ΔKd (N vs. Ntl) is ~ 6-fold] and that the presence of HMGB1 exerts a similar effect on the Kd value that suggests, but does not prove, that the histone tails may be the target for the HMGB1 effect on ERα binding.

3. Discussion

3.1. The binding characteristics of the estrogen receptor suggest it requires a broader classification

Hormone receptors are ligand-induced transcription factors that are characterized by 1) the form they use to bind to DNA (homo- or heterodimers or monomers), 2) the nature of their DNA binding sites (inverted repeat or a direct repeat) and 3) the spacer between the half-sites. It is well established that the steroid hormone receptor, ERα, binds as a dimer to an inverted repeat of 5'-AGGTCAxxxTGACCT-3', having a three base spacer. As a result of these characteristics, it has been logical and convenient to group ERα as a steroid hormone receptor (Class I) in the nuclear hormone receptor superfamily. However, the collection of new findings [4,6,38,43] show a much less restrictive DNA binding specificity and transcriptional activity, well beyond those that originally established the current classification. ERα has been shown to bind to inverted repeats, even some that do not have a 3 bp spacer. They bind to direct repeats with a variety of spacers, in addition to an everted repeat. Some of these arrangements are documented in promoters or enhancers considered important to estrogen responsiveness [44]. These findings suggest that ERα not only exhibits characteristics of steroid hormone receptors, but also those of some of the non steroid receptors. For example, the thyroid hormone receptor binds to the cERE(0) that contains no spacer ($n = 0$). Data show that ERα binds to this sequence also [5,38] and as a result, it is reasonable to suggest that the thyroid and estrogen receptor may compete for this common site in some tissues. Such a competition has already been documented [45,46] for thyroid receptor, TPα1, and although the mechanism remains unclear, this is consistent with the proposal of a competition, or receptor crosstalk, between receptors for a common response element. In addition, the consensus half-site for ERα binding is 5'-AGGTCA-3', the same as those of the Class II non steroid receptors, which bind direct repeats. On the other hand, this consensus sequence differs from those of the other Class I steroid receptors. Recognition of these alternative response elements for ERα and the finding that estrogen and thyroid hormones alter the effects of each other in specific cells suggests that there may be a significant cross-talk between the nuclear hormone receptors. These data suggest that ERα is a nuclear hormone receptor that has properties that overlap, or bridge, both the Class I and II receptors and should not be restricted into a single Class, Class I. As has been noted previously, the receptor activity at a promoter or enhancer sequence may be highly regulated by the recruited coactivators. The influence that HMGB1 may exert on these receptor interactions at common response elements can be expected to require further clarification and be of further interest.

3.2. HMGB1 restructures the canonical nucleosome in an ATP-independent manner

Incorporation of DNA within a nucleosome restricts access of transcription factors to their response elements that will necessarily inhibit transcriptional activation. Experimental findings have led to proposals that chromatin remodeling complexes (CRCs) that exhibit ATPase activity can remodel the nucleosome and facilitate transcription factor binding [47-51]. Our findings extend the concept of altering the nature of the nucleosome structure, but in an ATP-independent manner, with HMGB1 perhaps being in the same general class as FACT [31]. By transient interactions, HMGB1 restructures the canonical nucleosomes (N) to drive the formation of two alternate nucleosome forms, N′ and N″. ERα binds strongly to the cERE in both restructured nucleosome forms, with a Kd = 52 nM. In contrast, ERα has little affinity to the cERE in the canonical nucleosome, N, (Kd ~ 300 nM). In

addition, since there is no evidence that HMGB1 exhibits an ATPase activity, its action in the formation of N′ and N″ is ATP-independent. This being the case, HMGB1 and FACT, that contains an HMG box component, currently appear to be the only factors that exert their restructuring action in an ATP-independent manner.

In further support of the finding that HMGB1 restructures the nucleosome and facilitates transcriptional activation, it has been shown by chromatin immunoprecipitation (CHIP) assay that after estrogen treatment in MCF-7 cells, histone H1 is displaced from the single nucleosome that contains the ERE associated with the pS2 promoter and is replaced by HMGB1 to facilitate transcriptional activation. This indicates that HMGB1 plays an essential function in the ER-mediated transcriptional activation at the pS2 promoter [17].

As pointed out previously, it is important to emphasize that both restructured nucleosome forms (N′, N″) are stable at − 20 °C for months, which should allow a more comprehensive characterization. In contrast, the proposed intermediates or remodeled nucleosomes formed by all the reported chromatin ATPase complexes are unstable, short-lived species (half-life of minutes), which has hampered their characterization [52-55].

3.3. HMGB1 may enhance ERα/cERE binding and transcriptional activity in nucleosomal DNA by alternative mechanisms

HMGB1 most likely utilizes multiple avenues to restructure the nucleosome. HMG1 binds transiently in the minor groove of DNA and increases the flexure in the DNA [16]. This weakens the DNA/octamer binding surface to increase the conformational space of the nucleosome and aid in ERα gaining access to the cERE. In addition, ERα binds optimally to the cERE when the cERE is bent toward ER [56,57]. However, although our DNA construct positions the major grooves of the cERE facing outward for optimum ERα binding in the nucleosome, the cERE is naturally bent in the opposite direction, due to its curvature as it interacts with the histone octamer. Because of the flexure induced in the DNA by HMGB1, the bend in the cERE DNA may fluctuate more extensively to give ERα a more advantageous time frame to capture the more favorable binding configuration. An additional avenue for HMGB1 action is for it to electrostatically interact with the exposed tails of the core histones and/or the DNA. This will decrease the residence lifetime of the histone tails with the exposed nucleosomal DNA and permit ERα greater access to the cERE. In support of this, NMR findings show that the N-terminal domains of H3 and H4 are tightly bound to the DNA in the core particle up to ~ 0.35 M NaCl [58], which is in line with our competition data with NaCl. In the absence of HMGB1, the Kd for ERα binding to the tailless nucleosomes is 45 nM. This is comparable to the Kd (52 nM) for ERα binding to the HMGB1-restructured nucleosome and consistent with the core histone tails playing a significant role in inhibiting ERα/cERE binding in nucleosomal DNA.

3.4. A model for HMGB1 restructuring of the nucleosome and nucleosome dynamics

Overall, the HMGB1 interaction weakens multiple constraints in the octamer/DNA. This mechanism of action can be viewed in a manner similar to that proposed by Perutz for O2 binding to hemoglobin[59]. The canonical nucleosome can be viewed as a "tense" form of the nucleosome that, because of the octamer-DNA forces, is both structurally rigidly and functionally constrained. The interaction of HMGB1 disrupts a number of these forces, certainly electrostatic interactions between

the DNA and the core histones. With the restraints reduced, the nucleosome is converted into or takes on a "relaxed" form that is much more permissive to factor binding. This suggests that the nucleosome structure acts much like a "stress ball", in that its structure and function can be manipulated by the forces exerted on it in its immediate environment. As a stress is recognized, its structure changes, resulting in a change in the complexion of its functionality. This stress can come from changes in the immediate microenvironment, including pH, temperature, small molecule or protein interactions, posttranscriptional modifications and nucleosome condensation within a chromatin milieu. That being the case, one can speculate that, temporally, there will be an extensive ensemble of nucleosome states within the nucleus.

A concern about the activity of HMGB1, or any transcriptional coactivator that interacts with DNA, is how it targets the same specific response element, and at the same time, as the transcription factor. It is assumed that the coactivator is, more or less, uniformly distributed about the nucleus and has no visible mechanism to preferentially target the site of interest. A possible solution for HMGB1 in estrogen-responsive genes comes from GST-pull down experiments that showed that HMGB1 stably associates with ERα in the absence of DNA [60]. This indicates that HMGB1 could be chaperoned to the cERE by ERα, which would increase the level of HMGB1 at the promoter or enhancer site beyond the level that would occur by random, stochastic means. This mechanism would provide a synergistic mechanism for restructuring the nucleosome to facilitate stronger ERα binding and transcriptional activity as outlined with the pS2 promoter in MCF-7 cells [17].

An additional aspect of HMGB1 that may aid in transcriptional activation is that it was demonstrated that the C-terminus of HMGB1 interacts specifically with the glutamine-rich region (Q-tract) of the TATA-binding protein (TBP) [18]. If these multiple interactions of HMGB1 with ERα and the TBP in the transcriptional machinery can occur, it would clearly increase transcriptional activity.

It is also of note that the binding characteristics to DNA and nucleosomal DNA by the glucocorticoid receptor (GR), another steroid hormone receptor, are strikingly different than observed for ERα binding. GR binds strongly, and comparably, to the GRE in both DNA and in nucleosomal DNA [61]. This indicates that these two steroid hormone receptors exhibit very different capabilities to access and bind to ERE and GRE, respectively, within the nucleosome. This is also consistent with the finding that HMGB1 enhances the binding of GR to the GRE in mouse mammary tumor virus (MMTV)-DNA, but the influence of HMGB1 on binding in MMTV chromatin was minimal [62]. These comparisons add further support to the question of whether ERα should be strictly cataloged as a Class I nuclear receptor.

## 3.5. Nucleosomes can occur in a dynamic ensemble of structurally and functionally different structures

Physiological changes require that the regulation of gene expression be dynamic and require continual changes in chromatin structure. Fundamental to this is the restructuring of nucleosomes in chromatin by remodeling complexes, protein interactions and the changing microenvironment within chromatin.

Data were presented that showed that a distribution of nucleosome conformers (conformational isomers) is simultaneously present in equilibrium under conditions of varying NaCl and DNA concentrations. The various populations, their levels and the energy barrier between the conformers are sensitive to, and dynamically controlled, by the immediate microenvironment and the interaction

with HMGB1 on this nucleosome energy landscape. The binding of HMGB1 to the canonical nucleosome can be regarded as a "ligand-driven" conformational selection of states, or alternatively, a population shift on the energy landscape.

Figure 2A symbolizes a limited picture of two hypothetical energy landscapes for the three conformers. Using the conventional procedure to prepare the canonical nucleosome, landscape I shows N to be the predominant species, with N′ and N″ being kinetically trapped, and EMSA unable to detect the population of N′ and N″. Incubation of the canonical nucleosome with 1600 nM HMGB1, followed by gradient purification, leads to the conversion of N to N′ and N″ (path 3).

When HMGB1 interacts with the canonical nucleosome, it perturbs the distribution in the equilibrium of states. This drives a population change in the ensemble of nucleosome conformations, with energy landscape I converting to II. In state II, the population of N′ and N″ are the dominant populations, with the level of the canonical nucleosome greatly reduced. Figure 2B considers the canonical nucleosome, N, to be a "tense" conformer due to the structural constraints, many electrostatic in nature. As a result, ERα is not able to bind to the cERE (path 1). On exposing the nucleosome to 400 nM HMGB1, many constraints are reduced and the nucleosome is in a "relaxed" conformer, which facilitates more accessibility and flexibility in the ERE and ERα/ERE binding occurs (path 2).
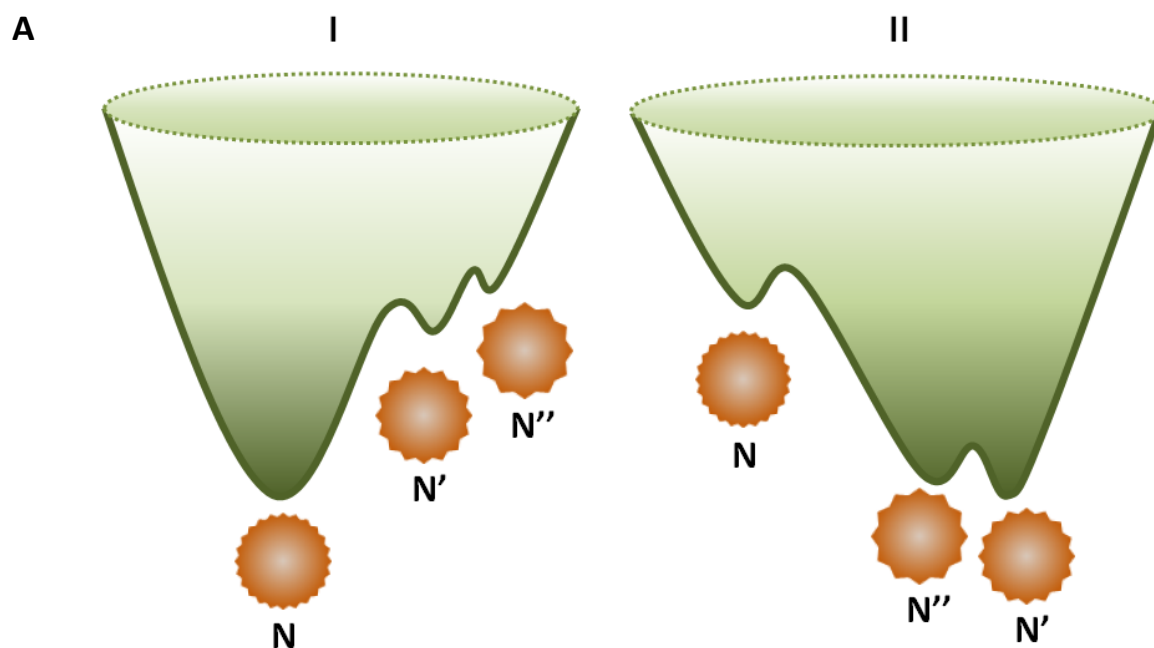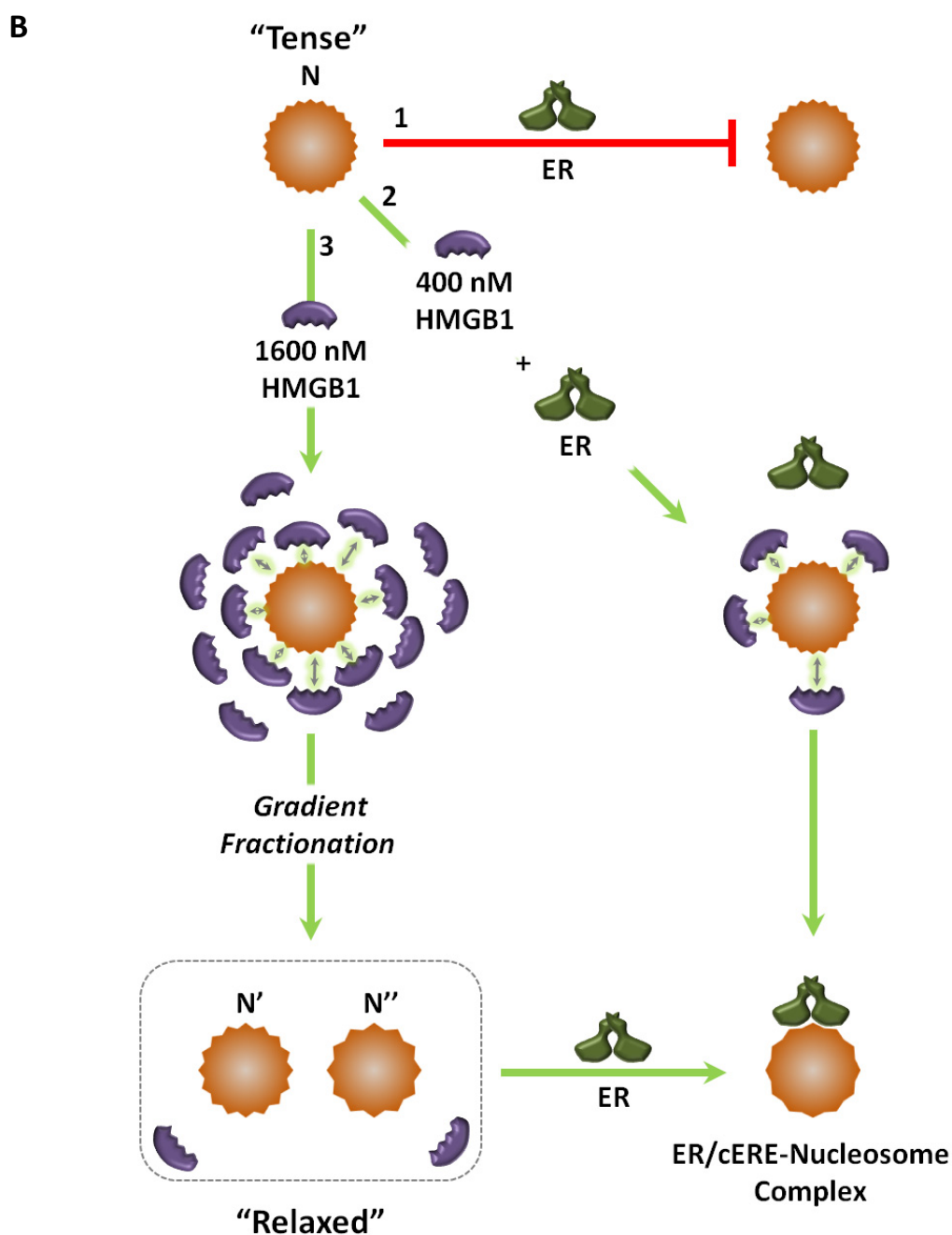
Figure 2. HMGB1 relaxes the canonical nucleosome structure and facilitates ER binding [40].

(A) Energy landscapes for canonical (I) and HMGB1-restructured nucleosomes (II). A hypothetical representation for the energy landscape of the canonical nucleosome, N, and the HMGB1-restructured nucleosomes, N′ and N″. Using conventional isolation protocols, the canonical nucleosome, N, is the predominant and thermodynamically most stable conformation. N′ and N″ are in low abundance, higher energy conformational isomers that are kinetically trapped near the bottom of energy landscape I. HMGB1 interaction with N reduces intranucleosomal

constraints, which resets the energy landscape (II), resulting in a population shift in which the N population significantly decreases and the population of the more "relaxed" and accessible N′ and N″ states increases. The more unstable form, N″, sets in a shallower potential well than that for N′. Although interactions with HMGB1 provide the driving force to restructure N into these states, these forms remain stable and although in equilibrium with the canonical state under many solution conditions, can revert to the canonical nucleosome on challenge with increasing concentrations of NaCl and DNA.

(B) Interaction of the nucleosome with HMGB1 and ER. The canonical nucleosome (N) represents a "tense" and relatively inaccessible conformational isomer (pathway 1) and ER does not bind to the canonical nucleosome state. In the presence of 400 nM HMGB1 (pathway 2), due to the transient and dynamic "hit and run" interaction of HMGB1 with the nucleosome, represented by arrows (↔), the intranucleosomal constraints are relaxed, which facilitates ER binding. ER binds to the nucleosome to form ER/cERE nucleosome complex. In the presence of 1600 nM (pathway 3), the intranucleosomal constraints are relaxed due to increased "hit and run" interaction of HMGB1. After gradient fractionation, the restructured nucleosomes (N′ and N″) are isolated and contain only low (25 nM) levels of HMGB1 which maintain the more accessible and "relaxed" conformational isomers (N′ and N″) that permit ER binding.

The paradigm of a statistical ensemble of nucleosome conformers on an energy landscape, with the "ligand", HMGB1, interaction changing the population of the ensemble, can be considered a first level approximation of what may occur within the nucleus. The methylation of DNA, post transcriptional modification of the histones, mutations in the DNA and especially the level of nucleosome compaction within the chromatin milieu—all these factors would suggest that there will be a virtual spectrum of nucleosome conformers at any time, which will fine tune the functional activity of the genome.

4. Conclusions

1. Estrogen receptor α (ERα) binds to the half-site, 5'-AGGTCA-3', and to this half-site in inverted, everted and direct repeats that have variable spacers.
2. HMGB1 protein further facilitates the extent of DNA binding to these non-conventional estrogen response elements (EREs).
3. ERα utilizes these non-conventional EREs to drive transcription within the U2OS cell line.
4. There is growing evidence for a possible cross-talk between ER and the Class II nuclear hormone receptors at the DNA level since they share the same common half-site (cHERE) in their response elements.
5. ERα does not bind to the cERE within a rotationally phased and translationally positioned nucleosomal DNA.
6. HMGB1 alters the structure of the canonical nucleosome to facilitate strong and sequence-specific ERα binding to the cERE.

7. HMGB1 utilizes a "hit-and-run" mechanism in its transient interactions with DNA and the nucleosome.

8. The interaction of HMGB1 with nucleosomes begins to reveal the dynamic nature of the ensemble of nucleosome structures as it restructures the "tense" canonical nucleosome into more "relaxed" and permissive nucleosome states that facilitates ER binding and, in collaboration with other regulatory factors, plays a role in transcriptional activation of estrogen-responsive genes.

Acknowledgments

Conflict of interest

None declared.

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