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Hypothesis

Histone H1 and the regulation of transcription by nuclear receptors

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Histone H1 is a eukaryotic repressor which recognizes specific DNA structures, and nucleotides regulate its interaction with DNA. Since their mode of action may be considered similar to that observed in the case of plasma membrane GTPases, H1 may be regarded as an ATP/GTPase involved in the action of nuclear receptors. A hypothesis is put forward here to suggest that transcriptional activators CTF/NF-I and AP-1 (*fos/jun*), for example, are effectors for H1. H1 and CTF/NF-I may be members of a stimulatory regulatory cascade for nuclear receptor action that ends with selective activation of chromatin through histone modification and the disruption or a more subtle structural change of a specific nucleosome, while an opposite effect may be obtained through modification of *fos/jun* by H1.

Histone H1; Nuclear receptor; CTF/NF-I; AP-1; Nucleosome; GTPase

1. INTRODUCTION

DNA in eukaryotes is packed into nucleosomes [1] which consist of two turns of DNA twined around pairs of histones H2A, H2B, H3 and H4, and histone H1 binds to the spacer/hinge region [2], a site between the entering and leaving DNA strands. H3 and H4 differ from H2A and H2B with respect to their evolutionary origin, and H2A and H2B may be envisaged as being deposited on them, obviously bending the DNA in a similar manner to prokaryotic HU proteins [3].

A novel type of DNA–protein interaction has recently been characterized for histones [4], in which recognition of an exceptionally narrow minor groove of A+T-rich DNA regions takes place through SPKK motifs in their mobile tails. The SPKK motifs in many of the core histones are subject to acetylation [5] and phosphorylation [6], and the presence of these modifications correlates with chromatin activation [5]. Since acetylation evidently impairs the chromatin condensation properties of the histones [7] although it does not affect DNA binding (Oikarinen, J., Mannermaa, R.-M., Tarkka, T., Yli-Mäyry, N. and Niemelä, O., unpublished results) or nucleosome positioning [8], it has been suggested that the introduction of this reversible modification may release the inhibition brought about by chromatin condensation [9], allow transcriptional initiation complexes access to the start site [10] and render the nucleosomes negotiable by RNA polymerase upon the transcription activation process [11]. On the other hand, phosphorylation of the histones at SPKK motifs has been suggested to impair their DNA binding, and this modification may thus also be of regulatory importance [6] (Nilsson, P., Thornell, A., Oikarinen, J. and Grundström, T., unpublished results).

A hypothesis is put forward here to explain how nuclear receptors, and some other transcriptional regulatory proteins, may be involved in the regulation of the posttranslational modification reactions of histones, and act through producing changes in the chromatin structure.

2. HISTONE H1 AS A NUCLEOTIDE-BINDING PROTEIN

The mode of H1 interaction with DNA and the nucleosome is currently under intense investigation. A number of SPKK motifs in the charged, basic C-terminal tail are thought to direct its binding to the A+T-rich nucleosome spacer regions [12]. Several pieces of evidence suggest preferential binding of H1 to CTF/NF-I [13–15] and AP-1 sites on DNA (Mannermaa, R.-M., Yli- Mäyry, N., Tarkka, T. and Oikarinen, J., unpublished results), modulation of the function of these regulatory proteins thus being an intriguing target for the H1 action.

It is evident in the light of recent data that H1 contains a phosphate and nucleotide binding site [16], which interacts preferentially with GDP/GTP and ADP/ATP [17,18], so that these nucleotides affect H1 DNA binding [19]. Nucleoside diphosphates are thought to stabilize a state in which H1 binds better to

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DNA, while nucleoside triphosphates impair its binding [18]. H1 is also able to hydrolyze nucleoside triphosphates to nucleoside diphosphates, and incorporate phosphate to exogenous proteins [17]. These findings are of utmost interest when extrapolated to the current knowledge on the mechanisms of the regulation of transcription.

Consistent with the above results, the H1 molecular structure displays considerable similarity to that of GTPases [18], presence of the loops involved in the recognition of guanine and the hydrolysis of the GTP β-γ phosphate bond by *ras*-p21 being demonstrable (Yli-Mäyry, N., Tarkka, T. and Oikarinen, J., unpublished results). In the light of the above findings, H1 may be considered the nuclear ATP/GTPase involved in mediating the action of nuclear receptors [20] (Fig. 1).

3. CTF/NF-I COUNTERACTS CHROMATIN FORMATION

CTF/NF-I is a eukaryotic DNA-binding protein which is involved in both DNA replication and gene transcription [21], recognizing preferentially the 5′-TTGGCAnnnTGCCAA-3′ sequence with the perfect dyad of symmetry [14]. The motifs recognized by H1 and CTF/NF-I may be similar in appearance [14], although the exact consensus sequence for H1 binding sites remains unelucidated, and the two proteins may be parts of a regulatory system with a common or overlapping specificity for certain sites on DNA. Several forms of CTF/NF-I are synthesized in cells, due to differential splicing of primary transcripts arising from a single gene or multiple genes displaying heterogeneity in their C-terminal parts and in a region within their N-terminal DNA-binding domains [22,23].

The exact mechanism of CTF/NF-I action is not known, although this protein is evidently capable of counteracting the packing of DNA into chromatin [24]. A number of functionally important regions have been identified in CTF/NF-I, comprising the N-terminal DNA-binding domain which is per se responsible for the activation of DNA replication and a C-terminal Pro-rich transcription activation domain [25]. Interestingly, the CTF/NF-I primary structure displays marginal homology with protein kinases and bacterial chloramphenicol acetyltransferases (see [26]), and the predicted molecular structure has revealed considerable conservation of the regions that had been shown to be responsible for the catalytic activity of the acetylases.

4. AP-1 (*fos/jun*) AS A TRANSCRIPTIONAL ACTIVATOR

AP-1, the heterodimeric complex of various forms of *fos* and *jun* [27], is a transcription activation protein involved in mediating the effects of a number of effectors such as phorbol esters and TGF-β on gene expression [28]. Various forms of *fos/jun* contain a highly homologous DNA binding domain and they recognize a common consensus DNA sequence, 5′-TGA(C/G)TCA-3′. AP-1 belongs to a larger family of transcriptional activator proteins characterized by a coiled-coil leucine zipper dimerization domain and a basic DNA-binding domain in the N-terminal end of the coiled-coil [29].

AP-1 takes part in the regulation of the expression of a number of cell proliferation-associated genes such as those for various extracellular proteinases [28,30]. Increased production of various collagenases, this being a group of the proteinases, is required for malignant growth and metastasis in neoplastic diseases, and *fos* and *jun* are per se capable of inducing their synthesis and transforming the cells when produced in excess. The aberrant expression of the collagenase genes in transformed cells, as well as the induction during the normal cell division, is blocked by an administration of appropriate steroid hormones. The mechanism of the inhibition has remained unrevealed to date. Since several mechanisms have been demonstrated to be responsible for the regulation of AP-1 activity, including modulation of the synthesis of *fos* or *jun*, phosphorylation of the proteins and binding of specific inhibitors, the mechanism of the steroid interference with the AP-1 action may turn out to be complex [31,32].

5. MODE OF ACTION OF NUCLEAR RECEPTORS

In general, nuclear receptors exert their effects through binding to DNA [33]. A ligand such as steroid hormone, T_3, retinoic acid or 1,25-dihydroxycholecalciferol is needed for this interaction. The ligand enables dissociation of the receptor from depository hsp90 [34] and dimerization [35]. Nuclear receptors evidently interact in addition to hsp90 with other heat shock proteins such as hsp70. The significance of these interactions remains unelucidated although they may be envisaged as taking part in nucleotide exchange, as discussed below. Upon binding to DNA, nuclear receptors are thought to bring about a change in the chromatin structure that results in induction or repression of certain genes [36–38]. The mechanisms by which the induction is brought about are unknown, although they may involve disruption or a more subtle structural change in a specifically positioned nucleosome in the promoter region of the inducible gene (Fig. 2) [36,38]. These changes may be accompanied by changes in histone acetylation [39].

It has recently been suggested that nuclear receptors may act through mechanisms similar to those of the GTPase-coupled receptors on the plasma membrane (for details see e.g. [40]). Considerable homology and conservation of the regions responsible for the interaction of the plasma membrane receptors with GTPases

was observed in the nuclear receptors, thus suggesting analogous mechanisms of action and a common evolutionary origin for the two receptor families [20].

6. REGULATORY ROLE OF HISTONE H1

H1 is thought to act as a eukaryotic repressor [41]. It triggers nucleosome aggregation to solenoid structures, characteristic of the repressed heterochromatin, with six nucleosomes per turn and H1 inside the ordered structure. On the other hand, a number of experiments suggest that H1 is more loosely bound to chromatin in active areas than in the areas not as efficiently expressed, although it is generally thought to be still present [42,43]. It is of interest to speculate how all the above findings concerning the regulation of the eukaryotic gene expression by nuclear receptors, CTF/NF-I and AP-1 may be put together.

The presence of an intact CTF/NF-I binding site is an absolute requirement for the steroid action in the case of MMTV-LTR [36–38], and this site resides in the nucleosome hinge/spacer region, corresponding to the site to which H1 binds in the chromatosome [44]. There is increasing evidence that this may also be the case with other nuclear receptor-inducible genes. Since the binding of steroid receptors to corresponding responsive elements results in a change in the structure of this nucleosome in particular (Fig. 2) [38], it may be assumed, by analogy to the mode of action of the receptors on the plasma membrane (see [40]) that nuclear receptors cause dissociation of H1, the ATP/GTPase, from the nucleosome. Consistent with this, differential DNA binding of H1 has recently been demonstrated upon GDP/GTP exchange [45], this being in agreement with the suggestion that nuclear receptors may act by enabling the nucleotide exchange in H1 and thereby impairing the H1 affinity to DNA [19].

It has very recently been demonstrated in a number of studies that nuclear receptors counteract the effect of AP-1 on transcription and vice versa [46–48]. It is evident from these experiments that the effects are not due to binding of the two protein families to the same DNA element or to overlapping sequences, and the counteraction of the AP-1 action by steroid receptors actually does not necessarily require the binding of the steroid receptor to DNA. The effects may be brought about by mutual interaction of the two proteins or by that through a third protein factor [48].

The inhibition of AP-1 action by nuclear receptors may involve phosphorylation of *jun* at Ser in an SPKK motif homologue located towards the amino terminus from the coiled-coil dimerization/DNA-binding domain and thought to enhance DNA binding of *fos/jun* in a cooperative manner [32]. This site of *jun* becomes dephosphorylated upon activation of the protein kinase-C pathway. The introduction of phosphate to the SPKK motif blocks DNA binding of AP-1 [49] and H1 [6], and

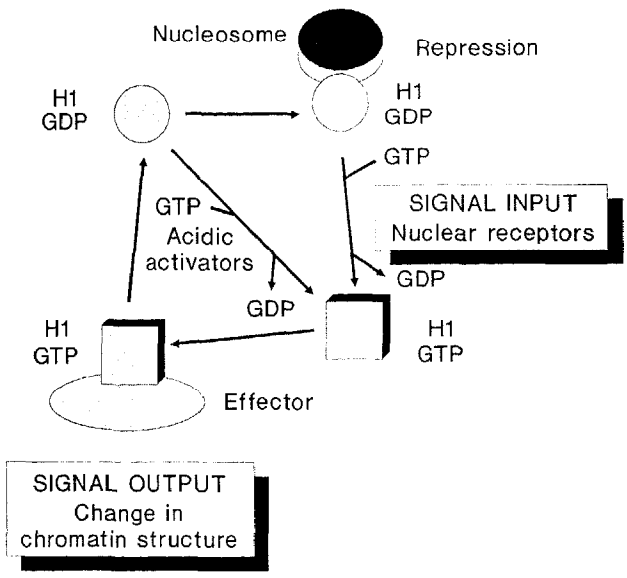

Fig. 1. Schematic representation of histone H1 as an ATP/GTPase. A hypothesis is presented here to suggest that histone H1 is a nucleotide binding protein [18], and that GDP/GTP exchange in H1 is facilitated by nuclear receptors (*signal input*) [45]. Upon binding of GTP H1 is incapable of binding to DNA [19], and it may interact with effector proteins (*signal output*). H1 itself, or in the presence of auxiliary proteins, is capable of hydrolyzing nucleoside triphosphates to corresponding diphosphates [17]. H1 binds to DNA in the presence of the nucleoside diphosphate [18]. Acidic activator proteins may facilitate the hydrolysis or nucleotide exchange and produce thereby chromatin activation [45].

introduction of this modification may thus cause the inhibition of AP-1 activity upon the steroid treatment. It is of interest in this context that H1, the mediator of the nuclear receptor action, is capable of hydrolyzing nucleoside triphosphates and phosphorylating exogenous proteins. The SPKK motif may, at least in some situations, be a target for this modification.

7. ARE CTF/NF-I AND *fos/jun* EFFECTORS FOR HISTONE H1?

In conclusion, several pieces of evidence exist that nuclear receptors bring about changes in the chromatin structure, these changes possibly being responsible for the alterations in the DNA template activity. The recent findings imply that H1 may be involved in triggering the changes, and the effector proteins involved in the putative ATP/GTPase action of H1 need to be elucidated. When the mode of H1-DNA interaction is taken into consideration, H1 is expected to bind to the opposite side of the DNA double helix when compared with CTF/NF-I and *fos/jun* which mainly interact with the DNA major groove. This should allow concomitant binding of H1 and one of the latter two, to close contact, and enable mutual interference with the functioning of each other.

It is even more intriguing to postulate that, upon the

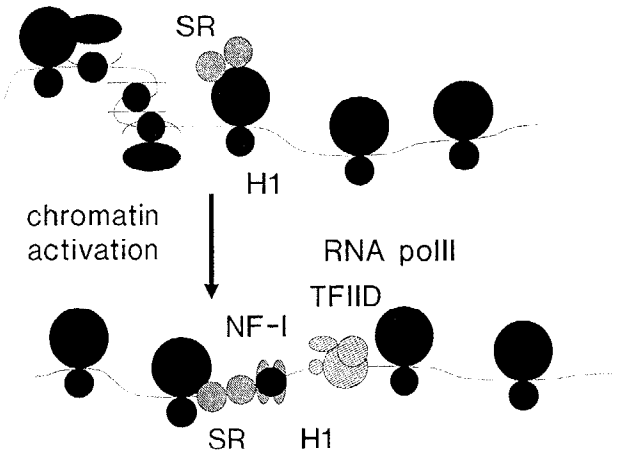

Fig. 2. Steroid receptor-induced disruption/modification of a specific nucleosome during chromatin activation. Nuclear receptors such as those for steroid hormones (*SR*) bind to DNA, and exert their effects through a specifically positioned nucleosome (regulatory nucleosome). The binding of the receptor to a responsive element may result in a dissociation or more likely in a structural change in the regulatory nucleosome, and is assumed to counteract chromatin condensation brought about by nucleosomes and histone H1. *CTF/NF-I* may be needed in the process. Subsequent revelation of the transcriptional start site allows binding of auxiliary transcription factors to the promoter (*TFIID*).

nuclear receptor facilitated nucleotide exchange (Fig. 1), H1 activates CTF/NF-I, a histone acetylase directed to specific sites on DNA (see [26]), since this process would result in selective acetylation of the nucleosome core histones, interference with their chromatin condensing capacity and the revelation of a transcriptional start site (Fig. 2). Furthermore, there is increasing evidence that nuclear receptors may be immediate mediators of the growth factor action, changes in the receptor activity possibly affecting H1 function and the chromatin structure. In agreement with this, a CTF/NF-I binding site mediates the stimulatory effect of TGF-β on the mouse α_2(I) collagen gene expression, H1 interacts with this site, and the gene is positively regulated at the level of transcription by steroid hormones.

On the other hand, in the case of inhibition of transcription, phosphorylation of the SPKK motif of *fos/jun* may take place, and should lead to impaired DNA binding and interference with the transcriptional activation brought about by that protein. This may ultimately lead to a transcriptional block through induction of the repressed chromatin state, as suggested previously. Consistent with this, an AP-1 site mediates the inhibitory effect of TGF-β on the transin gene expression, H1 binds to this site (Mannermaa, R.-M., Yli-Mäyry, N., Tarkka, T. and Oikarinen, J., unpublished results) and the gene is negatively regulated at the level of transcription by steroid hormones. The two mechanisms put forward above would thus, at least partly, explain the inhibitory and stimulatory effects of a number of extracellular effectors on gene expression, and those brought about by the binding of nuclear receptors to their responsive DNA elements.

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