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Role of Chromatin Structure in Transcriptional Regulation of MMTV LTR Hormono-Dependent Promoter

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Studies of chromatin structure were performed in mouse fibroblast cell lines containing Bovine Papilloma Virus (BPV) based artificial minichromosomes containing Mouse Mammary Tumor Virus (MMTV) Long Terminal Repeat (LTR), a retroviral promoter regulated by glucocorticoids, driving the transcription of v-Ha-*ras*. These minichromosomes fractionate with the «active chromatin», indicating an association of the minichromosomes with components of the «nuclear matrix». Two regions of the minichromosomes upstream and downstream of v-Ha-*ras* are involved in this interaction. MMTV LTR promoter is associated with nucleosomes precisely positioned on the DNA sequences. Hormonal activation is accompanied by a structural change of the nucleosome associated with the hormone response elements (HREs). This structural change can be visualized by the appearance of a hormono-dependent DNaseI hypersensitive site. Anti-hormones, even when able to promote a strong binding of the receptor to the nucleus, are unable to induce the chromatin structural change. The strong association of the hormone-receptor complex with the nucleus is necessary to induce the DNaseI hypersensitive site and to maintain the transcription, but is not necessary for DNaseI hypersensitivity maintenance. This suggests a double role for the hormone-receptor complex: 1) induction of a chromatin rearrangement and 2) transcriptional transactivation.

Key words: Chromatin, Steroids, Transcriptional activation.

Gene regulation by steroid hormones involves interaction of the «activated» hormone-receptor complex with target DNA sequences located in regulated promoters (16, 22). In eukaryotes, DNA is intimately associated with histones. This organization may considerably limit the accessibility of a large portion of the genome to diffusible regulatory proteins.

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DNA sequences characterized as regulatory targets are usually nucleosome free, and can be detected by their hypersensitivity to DNaseI (4,5). Precisely positioned nucleosomes have been demonstrated on a variety of putative gene regulatory regions (4, 14, 17). Their role in transcription regulation is still poorly understood. However, their organization is most often disrupted concomitantly with gene activation. The mechanism and the extent of rearrangements have not yet been elucidated.

The hormono-dependent MMTV LTR promoter has been extensively studied. It is a complex promoter under glucocorticoid regulation that also displays a tissue-specific expression. Both primary DNA sequence and conformation (chromatin structure) are involved in a set of positive and negative regulatory events. The roles of various regions of the LTR in transcription level control have been investigated by transient transfection assays and in transgenic mice (1, 10, 13, 25). It has been shown in transgenic mice that the distal part of MMTV LTR seems to be important for tissue-specific expression (25). Transient transfection studies have identified two regions as putative targets for negative regulatory factors (10, 13). However several questions have never been addressed: Are such elements functional in a situation in which the target sequences are established in the cell lines? Are they organized in a chromatin structure? Does the chromatin, under some circumstances, undergo structural changes?

In this paper we will discuss the role of chromatin structure in transcriptional activation of the hormono-dependent MMTV promoter.

Experimental system. — All the experiments described here have been performed in mouse fibroblast cell lines in which artificial minichromosomes have been established (15). The typical structure of a minichromosome is presented in figure 1. They contain the exogenous reporter gene v-Ha-*ras* driven by the MMTV LTR (hormono-dependent promoter) and the 69 % transforming fragment of the Bovine Papilloma Virus (BPV) that allows the establishment, as minichromosomes, of an amplified number of copies of the construct.

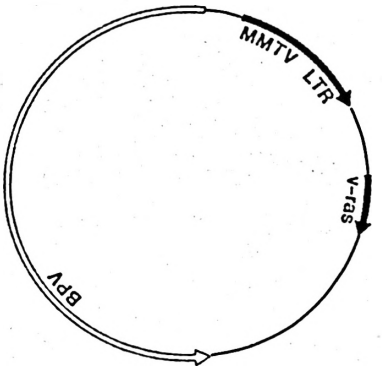

Fig. 1. *Map of the minichromosomes.*

Minichromosomes fractionate with active chromatin. — Active chromatin (containing genes actively transcribed or in such a differentiation stage that can be immediately turned on by a stimuli, e. g. hormone) and inactive chromatin (containing genes that cannot be transcribed) can be fractionated by centrifugation, from nuclei digested with micrococcal nuclease and lyzed in a low salt medium. Active chromatin is associated with the residual pellet (containing the nuclear matrix), while inactive chromatin is recovered in the supernatant. In cell lines containing the minichromosomes described above, 70 % of the bulk DNA is in the inactive chromatin fraction. On the contrary, v-Ha-*ras*, the reporter gene, is mostly present in the active chromatin fraction whether or not the cells were treated with the hormone (fig. 2). This suggests an interaction of the minichro-

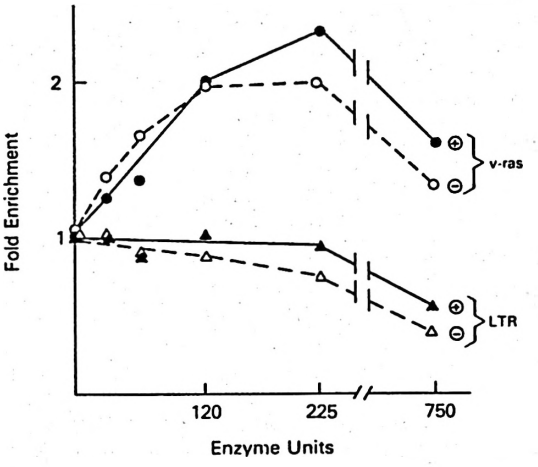

Fig. 2. *Distribution of MMTV LTR and v-Ha-ras in the active chromatin fraction.* Nuclei were digested with the indicated amounts of micrococcal nuclease. MMTV LTR and v-Ha-*ras* were measured in the active chromatin fraction by the dot blot technique.

mosomes with nuclear components, probably the «nuclear matrix». Such an interaction was investigated using *in situ* and *in vitro* approaches.

a) *in situ* experiments: nuclei were extracted with LIS (lithium diiodosalycilate) (12), leading to histone-depleted nuclei, digested with a restriction enzyme (HaeIII), and DNA released or associated with «scaffold» isolated by centrifugation.

b) *in vitro* experiments: nuclear matrices were prepared (2) incubated with end-labelled DNA fragments corresponding to the minichromosome, and DNA associated with the nuclear matrix was separated from free DNA by centrifugation.

The two approaches indicate a strong interaction of the DNA sequences located just upstream and downstream of the v-Ha-*ras* gene with the nuclear matrix. This interaction may play a role in establishing the transcription ability of the construct but is probably not directly related to hormonal regulation of the promoter.

MMTV LTR is covered with an array of positioned nucleosomes. — MMTV LTR is a target for hormone regulation. Association of DNA sequences with nucleosomes may play a role in regulation of gene expression by changing the conformation or accessibility of the DNA sequences, targets for regulatory factors. Experiments were performed by cleaving the DNA in the nuclei with micrococcal nuclease or with a chemical (MPE-Fe^{2+}) that cuts the DNA associated to nucleosomes preferentially in the linker region (20). The results of such experiments are summarized in figure 3. In the absence of hormone, MMTV LTR is covered with an array of positioned nucleosomes. The receptor target DNA sequence (GRE) seems to be wrapped around a nucleosome in the absence of hormone.

Glucocorticoid induces a conformational change of the nucleosome associated with the GREs. — Changes in chromatin structure, concomitant with hormonal activation of the promoter, were investigated using two different approaches: cleavage of the chromatin, in nuclei from cells treated or not with the hormone, by MPE-Fe^{2+} or DNaseI (20).

a) *MPE-Fe^{2+} cleavage.* In the nuclei from cells treated with dexamethasone, the DNA sequences containing the GRE (that are apparently associated with a nucleosome in the absence of hormone) become hypersensitive to the chemical (figure 3).

b) *DNase I hypersensitivity studies.* Nuclei from cells untreated or treated with increasing amounts of dexamethasone were digested with DNaseI. Hormone treatment induced a DNaseI hypersensitive site, located over the GRE (fig. 3). Figure 4 presents the quantitation of the autoradiogram by densitometric scan. The intensity of the hypersensitive site is re-

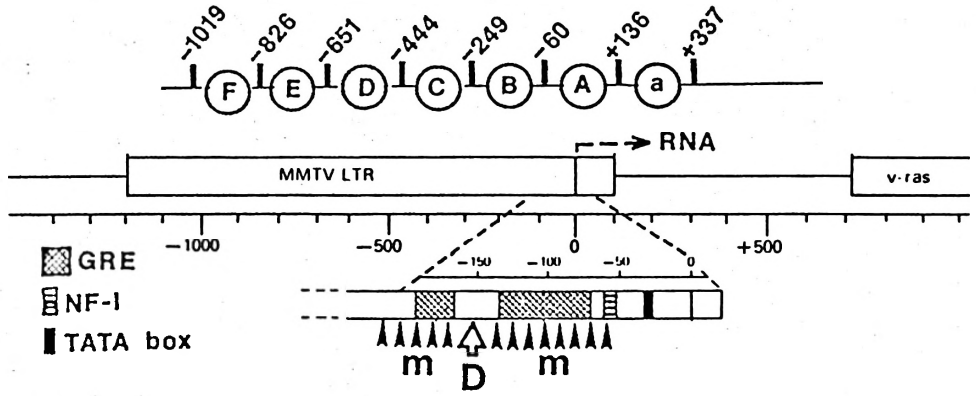

Fig. 3. *Chromatin organization of MMTV LTR.*
On the top of the MMTV LTR map is presented the nucleosome organization of the LTR. Underneath the enlarged area, containing the GRE and binding sites for NF-I and TFIID (TATA box), are figured the position of the hormonodependent DNaseI hypersensitive site (white arrow) and the region hypersensitive to MPE-Fe^{2+}, in the presence of hormone (black arrows).

lated to hormone concentration and can be super imposed on the dose-response curve for transcriptional activity assayed by nuclear run-on.

These data suggest that the interaction of the hormone-receptor complex with the GRE can be correlated with a local change in chromatin structure of this region of MMTV LTR.

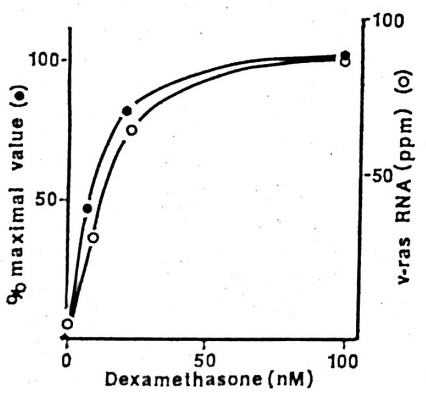

Fig. 4. *Comparison of MMTV LTR transcriptional activity and DNaseI hypersensitivity, as a function of hormone concentration.*

Anti hormones are unable to induce the conformational change of the nucleosome. — In the cell lines described here, dexamethasone-mesylate is a pure antagonist. It is also able to induce the nuclear transfer of the glucocorticoid receptor (24). In order to determine if the chromatin structural changes described above were the direct result of the interaction of the receptor with the GRE, we investigated the effect of the anti-hormone on DNaseI hypersensitivity. The antagonist, under conditions in which the receptor is efficiently translocated to the nucleus, is unable to induce the DNaseI hypersensitive site and, in the presense of hormone, prevents its hormono-dependent formation (21). These results suggest that the antagonist-receptor complex is able to interact with the chromatin, but unable to induce its conformational change, suggesting a critical role for this change in transcription stimulation.

Role of the hormone-receptor complex in maintenance of DNaseI hypersensitivity and transcription activation. — We followed the nuclear transfer of the hor-

mone-receptor complex upon treatment time with the hormone, in the cell lines containing v-Ha-*ras* as a reporter gene. In these cells, after a rapid uptake, the receptor-hormone complex was released from the nucleus. This suggests that synthesis of v-Ha-*ras* protein causes a change in intracellular receptor distribution. A similar observation has been made upon v-*mos* protein synthesis (19). This decrease in nuclear hormone-receptor complex can explain the negative effect, on glucocorticoid-regulated gene transcription, of an increased expression of several oncogenes, including v-Ha-*ras* and v-*mos* (9).

In order to investigate the role of the hormone-receptor complex in transcriptional activation, we have treated cells for various time periods with triamcinolone, measured the nuclear hormone-receptor content, and followed the DNaseI hypersensitivity. A maximal nuclear uptake of the hormone-receptor complex was achieved after 30-40 minutes, and then the nuclear receptor content decreased, to reach approx. 50 % of the maximal value at 2 hours. This pattern is similar to the transcription time-course pattern described by others (9). The intensity of the DNaseI hypersensitivity site increased with the increase of the nuclear hormone-receptor complex, but in contrast remained constant at its maximal value up to 2 hours of hormone treatment. These results suggest that 1) the hormone-receptor complex is necessary for the establishment of the DNaseI hypersensitive site, but not for its maintenance; 2) the presence of the DNaseI hypersensitive site is not sufficient to promote transcriptional activation.

Discussion

The organization of eukaryotic DNA in nucleosomes and the formation of higher order compacted structures allows the cell to solve the problem of packaging its DNA. The association of DNA, particularly regulatory regions, with core histone octamers can be critical, especially if nucleosomes are precisely positioned over such sequences. It has been demonstrated that DNA wrapped in a nucleosome is bent and kinked and the DNA helix is distorted (8, 14). These changes in DNA conformation may affect specific protein-DNA interactions. In addition, the intimate association of core histone octamers with DNA may produce steric hindrances, preventing regulatory factors from binding their targets. Association of DNA with a chromatin structure containing precisely positioned nucleosomes can also play a role in bringing into close vicinity DNA sequences normally at a large distance from each other, facilitating protein-protein interactions between DNA-binding regulatory factors. In several examples where core octamer positioning over a specific DNA sequence has been investigated, the phasing was dramatic, with cores positioned at a single base pair resolution (23). This reinforces the possible role of nucleosome positioning in gene regulation.

Transcription from MMTV LTR promoter is controlled by glucocorticoid in a multi-step process, involving binding of the hormone-receptor complex to its DNA target (GRE). The exact molecular mechanism of transcriptional activation remains to be elucidated. Binding of hormone-receptor complex to the GRE may facilitate the access or stabilize the interaction of other transcription factors with the promoter. It can also be directly involved in the formation of the transcription complex, contributing to the activation of the transcriptional machinery through protein-protein interactions.

In the absence of hormone, *in vivo*, MMTV LTR is associated with nucleosomes precisely positioned. The region containing the GRE seems to be wrapped in one of the nucleosomes. Reconstitution of an accurately positioned nucleosome

over that region has been successfully achieved *in vitro* and glucocorticoid receptor is able to recognize its DNA target associated with the histone core octamer (18).

The hormonal activation of transcription is accompanied by a local change of MMTV LTR chromatin structure. This change can be detected by the increased sensibility to chemical cleavage of the region containing the GRE and by the appearance of a hormono-dependent DNaseI hypersensitive site (7, 20, 26). In addition, the binding to the promoter of two transcription factors NF-I and TFIID, in a hormono-dependent fashion, has been demonstrated by *in vivo* exonuclease III footprinting (3). Binding of NF-I appears to be required for efficient transcription of the MMTV promoter after hormonal induction, since mutations in the NF-I binding site reduce hormonal response (1, 11). *In vivo* binding of NF-I to the MMTV promoter is detected only after hormone administration. This may result either from a stabilization of NF-I binding in the presence of the hormone receptor complex or be due to the inability of NF-I to access its DNA target in the absence of hormone. The NF-I DNA binding site is located at the limit of the nucleosome containing the GRE and can be inaccessible to the factor, unless the chromatin is rearranged. The nature of the hormonally-induced chromatin structural change over the GRE is not yet understood. It is apparently triggered by the interaction of the receptor with the GRE. But a productive interaction is only achieved when receptor is complexed with an agonist, the interaction with an antagonist-receptor complex being inefficient. The same line of evidence on action of antagonists has been obtained using recombinant mutated steroid hormone receptors (6). The chromatin structural change can result from the release of the nucleosome or from a change in its structure, weakening the strength of the interaction between DNA and core histone octamer. It is not known if the receptor must remain bound to the DNA after NF-I binding in order to maintain MMTV transcription. The preliminary results, presented here suggest that the hormone-receptor complex interaction with the GRE plays a double role in MMTV: change in chromatin structure and direct transactivation of the transcription machinery. This raises the question of whether or not chromatin structure by itself plays a general role in transcriptional regulation of hormono-dependent promotors, or if its role is limited to a few of them, such as MMTV LTR. Investigation of chromatin structure of other promoters will answer this question.

In the minichromosome model system that we have used, the reporter gene was present in the «active chromatin» fraction, whether or not the hormone was present. This distribution probably reflects more the «differentiation state» of the gene, rather than its transcription. It is probably not directly related to hormonal activation of the promoter.

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Resumen

Se estudia la estructura de la cromatina en líneas celulares (fibroblastos de ratón), que contienen minicromosomas artificiales establecidos con Virus de Papiloma Bovino (BPV) en los cuales el MMTV LTR (promotor del Mouse Mammary Tumor Virus, un retrovirus regulado por glucocorticoides) controla la transcripción de v-Ha-*ras*. Estos minicromosomas se fraccionan junto con la «cromatina activa», sugiriendo una interacción con componentes de la «matriz nuclear». Dos regiones del minicromosoma, «upstream» y «downstream» de v-Ha-*ras* están involucradas en la interacción. El promotor MMTV LTR está asociado con nucleosomas precisamente posicionados sobre las secuencias de ADN. La activación hormonal se acompaña de un cambio estructural del nucleosoma asociado con los elementos de respuesta

hormonal (HREs). Este cambio estructural se puede visualizar con la aparición de un sitio hipersensible a la DNasa I. Las antihormonas, aun siendo capaces de promover una interacción fuerte del receptor con el núcleo, no inducen el cambio de estructura de la cromatina. Es necesaria la asociación fuerte del complejo hormona-receptor con el núcleo, para inducir la hipersensibilidad a la DNasaI y mantener la transcripción, pero no lo es para mantener la hipersensibilidad a la DNasaI. Estos resultados sugieren un doble papel para el complejo hormono-receptor: 1) la inducción de un cambio de estructura de la cromatina y 2) la activación transcripcional.

Palabras clave: Cromatina, Esteroides, Regulación transcripcional.

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