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## **Control of gene expression by steroid hormones**

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**Summary.** The mechanism of action of steroid hormones involves their interaction with tissue-specific binding sites, and results in a precise modulation of gene expression. Both high-affinity receptors and secondary binding sites exist for steroid hormones in target tissues. Only steroid-receptor complexes were, in several cases, clearly shown to directly regulate transcription by interacting with DNA region(s) close to steroid-controlled genes. However other indications suggest that steroid hormones could also modulate transcription by altering chromatin conformation. These modifications encompass post-translational modifications of histones and non-histone proteins, as well as changes in the pattern of histone variants. Beside transcription, there are also evidences that steroid hormones can modulate gene expression by regulating some RNA processing events. Whether high-affinity receptors or secondary binding sites directly regulate these events is not known. These observations however suggest that several levels of control might exist for steroid hormones to precisely regulate gene expression.

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### **Introduction**

Steroids can be divided into several major classes, *e.g.* progestins, androgens, oestrogens, glucocorticoids, yet they share a common general mechanism of action. The lipophilic non-ionic character of steroid hormones allows them to be transported, mainly by simple diffusion across cytoplasmic membranes (Müller *et al.*, 1979). Although, in some instances, steroid binding proteins do exist within cell membranes (Giorgi, 1976 ; Pietras and Szego, 1977 ; Sadler and Maller, 1982), extracellular plasma carriers, together with intracellular binding sites, are generally considered the most effective regulators of cellular hormone levels (Giorgi, 1980). Once inside the cell, steroids can interact with a number of low affinity binding sites and eventually become metabolized by specific enzymes. Besides cytosolic steroid metabolism *per se*, steroid hormones might eventually modulate post-transcriptional processes (Liang *et al.*, 1977 ; Whelley and Barker, 1982 ; Cochrane and Deeley, 1984). However, the selective action of steroid hormones on a large variety of tissue-specific metabolic processes is mainly dependent on the presence of tissue-specific high-affinity receptors (Type I) or other binding sites (Type II) exhibiting lower affinity but larger capacity for steroids. Interactions of steroids with these binders allow them to control gene expression. In this article, after briefly summarizing an exponentially growing documentation on tissue-specific receptors and other binding sites, we have emphasized the important role the macromolecular organization of eukaryotic nuclei is likely to play for steroids to regulate gene

expression. Finally, our scope is to underline that multiple mechanisms of control might be involved for a precise modulation of gene expression by steroid hormones. Several excellent reviews, dealing in more detail with the binding properties of steroid receptors (Schmidt and Litwack, 1982 ; Housley *et al.*, 1984), or with DNA recognition sites for steroid-receptors (Groner *et al.*, 1984) were presented previously.

## **Steroid binding sites**

### **A. — High affinity receptors (Type I).**

Type I steroid receptors are characterized by a high affinity and a strict selectivity for a defined class of steroid hormones. Specific (Type I) receptors have been documented in a variety of tissues and for numerous species. Their presence is not confined to sex-related target tissues, as specific receptors are also present in liver (Eisenfeld *et al.*, 1980 ; Tamulevicius *et al.*, 1982 ; Bechet *et al.*, 1983, 1986b) and muscle (Michel and Beaulieu, 1980 ; Dahlberg *et al.*, 1981 ; Bechet *et al.*, 1986a).

cDNA clones for mRNA encoding receptors for glucocorticoids (Miesfeld *et al.*, 1985 ; Weinberger *et al.*, 1985) and for oestrogen (Walter *et al.*, 1985) have recently been isolated and the corresponding sequence of glucocorticoid receptor (Hollenberg *et al.*, 1985) and oestrogen receptor (Green *et al.*, 1986) have now been reported. Such data are likely to be determinant for a better understanding of the mechanisms of steroid binding and receptor activation and transformation.

#### **1. — *Binding of steroids to untransformed receptors.***

Several lines of evidence suggest that in the absence of steroid, unoccupied steroid receptors are present in different conformations, which differ in their ability to bind hormones (for review see : Schmidt and Litwack, 1982 ; Housley *et al.*, 1984). Some exist in an active binding state, others are unable to recognize their specific ligand. Transition to the active configuration(s) is believed to involve energy-dependent processes, such as sulfur reduction(s) or phosphorylation(s), in addition to other « endogenous factors » (Calkins *et al.*, 1976 ; Sato *et al.*, 1980 ; Leach *et al.*, 1982).

The cellular compartment(s) involved in steroid receptor inactivation is (are) not clearly defined. Receptor « *inactivating activity* » has been related to cellular membranes (Nielsen *et al.*, 1977), crude nuclear pellets (Auricchio and Migliaccio, 1980 ; Auricchio *et al.*, 1981a) and cytosol preparations (Sando *et al.*, 1979b). At least, some of the « *inactivating activity* » is insensitive to protease inhibitors (Nielsen *et al.*, 1977 ; Auricchio and Migliaccio, 1980). An ATP-dependent « *reactivating activity* » has also been partially purified from cytosol (Auricchio *et al.*, 1981b).

The bulk of the evidence supporting the view that some of these reactivating-inactivating entities involve a phosphorylation-dephosphorylation process, relies essentially on the demonstration that receptors for progesterone (Weigel *et al.*, 1981 ; Dougherty *et al.*, 1982), dexamethasone (Housley and Pratt, 1983 ; Singh and Moudgil, 1985) and oestradiol (Auricchio *et al.*, 1984) are phosphoproteins. In fact, part of the stabilizing effects initially ascribed to the phosphatase inhibitor, sodium molybdate (Sando *et al.*, 1979a ; Auricchio *et al.*, 1981a), was recently more satisfactorily explained as direct interaction with the untransformed receptor (Grody *et al.*, 1980 ; Housley *et al.*, 1984). Thus  $\text{MoO}_4^{2-}$  is capable of forming phosphomolybdate or sulfhydrylmolybdate complexes, which could prevent an irreversible loss of binding capacity (Housley *et al.*, 1984). An endogenous « *stabilizing factor* », detected in cytosol preparations, shares many properties with  $\text{MoO}_4^{2-}$  (Cake *et al.*, 1976, 1978 ; DiSorbo *et al.*, 1980 ; Leach *et al.*, 1982). *In vivo* this factor could stabilize the untransformed receptor and inhibit transformation of the receptor to the nuclear binding form (Sato *et al.*, 1980 ; Leach *et al.*, 1982 ; Housley *et al.*, 1984).

The second component which has pronounced effects on the steroid-binding capacity of untransformed receptors appears to be a reducing environment. In the absence of reducing agents (*e.g.* DTT), binding capacity is reversibly lost, even in the presence of  $\text{MoO}_4^{2-}$ . In some systems, such as rat liver cytosol, the « DTT effect » is hardly observed, due to high endogenous reducing activity (Leach *et al.*, 1982). Recent reports have tentatively identified NADPH-dependent thioredoxin as the endogenous « *activating factor* » which, by maintaining a reducing environment, would favour binding of steroid to the receptor (Grippio *et al.*, 1983, 1985 ; Housley *et al.*, 1984).

## 2. — Cellular localization of steroid receptors.

The cellular location of steroid receptors is at the moment, subject to some controversy (see Szego and Pietras, 1985). After decades of steroid research, the « two-step hypothesis » (Jensen *et al.*, 1968) emerged to become a dogma : receptors were cytoplasmic proteins which, upon binding to the steroid, were transformed into an « activated state » and then translocated to the nucleus. Recently, two approaches have, however, suggested an exclusive localization of oestradiol receptors in the cell nucleus, even without hormonal pretreatment. Greene & collaborators (King and Greene, 1984), using several monoclonal antibodies to oestrogen receptor, observed only nuclear immunoreactivity in a variety of target tissues. Welshons *et al.* (1984) adopted a different strategy and employed cytochalasin B to prepare enucleated cytoplasts. They also concluded that oestrogen receptors were purely nuclear entities. A compromise has already been proposed by Sheridan *et al.* (1979) who suggested that distribution of unbound receptors between cytoplasm and nucleus was determined by the water content of these compartments.

Obviously, only more data, relating steroid receptors to the dynamic processes of the cell, will resolve the issue of the « two-step hypothesis ».

Nevertheless, from wherever in the cell the untransformed receptor originates, there is general agreement on its reduced affinity for nuclei. *In vivo*, only the specific binding of a steroid to its receptor induces « transformation » of the steroid-receptor complex. Only this transformed complex binds avidly to nuclear structures, and is able to modify genetic expression. How transformation occurs is discussed in the next section.

### 3. — Transformation of the steroid-receptor complex into a nuclear binding state.

Cytoplasm and nucleus are inter-dependent and differentiate together. As all nuclear proteins are likely to be of cytoplasmic origin, the cytoplasm is potentially able to « reprogram » the nucleus, or at least to regulate nuclear functions. A selective localization of cytoplasmic and nuclear proteins requires an effective process to control the nucleocytoplasmic translocation of proteins to or from the nucleus. Transformation of steroid-receptors (Type I) might represent such a control mechanism. In conditions which exist *in vivo*, the steroid-receptor complex undergoes a rapid transformation to an « activated » (according to Litwack) or « transformed » (according to Pratt) state, characterized by its high affinity for nuclear structures (for review see : Schmidt and Litwack, 1982).

The transformation process can also be reproduced *in vitro* using various manipulations, such as high ionic strength, ammonium sulfate precipitation, elevated temperature, increased pH, gel filtration or dilution (Milgrom *et al.*, 1973 ; Redeuilh *et al.*, 1981 ; Mac Donald and Leavitt, 1982 ; Bodine *et al.*, 1984). The transformation of the steroid-receptor unit results in the exposure of positively charged regions on the surface of the complex (Milgrom *et al.*, 1973). This activated complex is then characterized and distinguished from the untransformed receptor by its preferential binding to polyanions, such as DNA (Rousseau *et al.*, 1975 ; Mac Donald and Leavitt, 1982), ATP-sepharose (Nishigori and Toft, 1980) or phosphocellulose (Mc Blain and Toft, 1983). Litwack and collaborators have developed a model according to which activation consists of both a dephosphorylation step and a step involving dissociation of an endogenous factor (the « stabilizing factor » of Pratt) from the untransformed steroid-receptor complex (Schmidt and Litwack, 1982).

No mechanism clearly details the subsequent nuclear processing or recycling of the receptor to the cytosol (Horwitz and Mc Guire, 1980).

### B. — Low affinity binding sites (Type II).

Besides the classical high affinity receptors (Type I), other steroid-binding sites (Type II), exhibiting lower affinity and specificity but higher capacity, occur in many tissues. Cytosolic Type II sites for oestrogens have been described in rat uterus (Clark *et al.*, 1978), rat liver (Dickson *et al.*, 1978), rat granulosa cells (Kudolo *et al.*, 1984a, b), chick oviduct (Taylor and Smith, 1982a), and guinea pig seminal vesicles (Weinberger, 1984). Such heterogeneity in types of hormone

binding sites is not limited to oestrogens and has also been observed for glucocorticoids (Barlow *et al.*, 1979 ; Do *et al.*, 1979) and progestins (Béchet and Perry, 1986).

Cytosolic Type II sites for oestrogens have been extensively studied in mature male rat liver (Dickson *et al.*, 1978 ; Eagon *et al.*, 1980 ; Miroshnichenko *et al.*, 1983). The levels in cytosol of these macromolecules change in relation to endocrine status. For example, they disappear from cytosol after castration or after oestrogen treatment of male rats (Dickson *et al.*, 1978 ; Eagon *et al.*, 1980) and can be induced in female rat liver by administration of androgens (Smirnova *et al.*, 1977, 1980).

There is no evidence that cytosolic Type II sites « translocate » to the nucleus (Eriksson *et al.*, 1978 ; Mataradze *et al.*, 1981 ; Taylor and Smith, 1982a ; Kudolo *et al.*, 1984a), and there has been no shortage of suggestions for putative roles for these binding sites. They have been implicated in a precursor-product relationship with Type I receptors (Taylor and Smith, 1982a). A role of « sink » or « sponge », protecting the cell against excesses of steroids has also been suggested (Dickson *et al.*, 1978 ; Eagon *et al.*, 1980). Alternatively, they might regulate the intracellular distribution and/or concentration of free steroid hormones (Kudolo *et al.*, 1984b) or protect steroids from being rapidly inactivated (Taylor and Smith, 1982a).

While « translocation » has not been demonstrated, the presence of Type II binding sites in nuclei has been shown, at least in rat uterus (Eriksson *et al.*, 1978 ; Clark and Markaverich, 1981) and rat liver (Béchet and Perry, 1986). Increasing interest in these nuclear Type II binding sites stems from two observations. First, though they do not seem to be translocated from the cytosol, they are induced, in some way, by hormonal pretreatment (Markaverich and Clark, 1979). Second, together with Type I sites, they do seem to play an important role in the events involved in oestrogen action (Markaverich and Clark, 1979 ; Markaverich *et al.*, 1981 ; Clark and Markaverich, 1981). Hormonal manipulations have indicated that Type II sites are more closely correlated with true uterine growth than Type I sites which are only transient entities within nuclei (Markaverich and Clark, 1979).

In *summary*, all those observations suggest that a primary role of steroid hormones is, by binding to Type I receptors, to induce the transformation of steroid-receptor complexes to a « nucleophilic » form. Steroids might also induce nuclear Type II binding sites. Both the occurrence *in situ* of Type II sites, and the high affinity of transformed steroid-receptor (Type I) complexes for nuclear structures, imply role(s) in the regulation of gene expression. In this regard, several lines of evidences indicate that steroid hormones could not only control RNA polymerase-dependent transcription, but also regulate the processing of pre-RNA to mature RNA.

## Steroidal control of transcription

### A. — General organization of chromatin.

The information which allows cells to differentiate or adapt to environmental stimuli is thought to be encoded in genomic DNA. Eukaryotic DNA is not found in discrete simple units. Genomic DNA, like most macromolecules, can undergo postsynthetic modifications. DNA is also packaged with histone proteins, and certainly interacts with many other nuclear constituents (specific proteins, RNP, nuclear skeleton,...). The occurrence of such complexes is not fortuitous, since eukaryotic gene expression takes place within a highly organized structure that allows specific genes to be recognized and properly phrased by relevant control systems. These must be as diversified or multifaceted as tissues can be specialized. In other words, accurate differentiation, tissue specificity and regulation of gene expression depend upon more complex « codes » superimposed on linear DNA sequences. The macromolecular organization of chromatin is therefore essential in determining what factors govern transcriptional activity, and particularly, how the same genetic code is differently expressed and controlled by steroid hormones in various tissues.

The basic unit for compaction of eukaryotic nuclear DNA is the « nucleosome core particle » which consists of 146 bp DNA coiled around a central protein core comprising one pair of each of the histones H2A, H2B, H3 and H4 (see Harauz and Ottensmeyer, 1985). With a further 20 bp of DNA adjoining the core, histone H1 seals two full turns of DNA around the histone octamer. This usual product of micrococcal nuclease digestion is termed the « chromatosome ». The « nucleosome » contains an additional « linker » DNA which connects neighbouring core particles. This latter fragment of DNA varies in length between species, tissues or even within the same cell (Allan *et al.*, 1980 ; Laskey and Earnshaw, 1980 ; Igo-Kemenes *et al.*, 1982 ; Thomas, 1983). This « beads on a string » nucleosomal chain (equivalent to the thin 100 Å fiber) provides the first level of chromatin organization. Further foldings of the chain generate higher levels of compaction, from thick (250 Å) fibers, to the loops or chromatin domains observed in interphase nuclei or metaphase chromosomes.

Thick fibers have been suggested as the basic structure for « inactive chromatin », and histone H1 seems to be essential for their formation (Thoma *et al.*, 1979 ; Thomas, 1984). Several characteristics of H1 might account for the dynamic properties of 250 Å fibers. H1 on its own can form homopolymers (« clisones » of Mc Ghee and Felsenfeld, 1980), and exchanges rapidly between segments of chromatin, even at physiological (0.1 - 0.2 M) ionic strength (Lasters *et al.*, 1981 ; Caron and Thomas, 1981 ; Louters and Chalkley, 1985).

Higher orders of organization involve the compaction of thick fibers into domains of chromatin (Benyajati and Worcel, 1976). According to the « domain model » (Murray and Davies, 1979 ; Lepault *et al.*, 1980), chromatin is precisely

organized into loops, anchored to a proteinaceous scaffold commonly termed nuclear matrix or skeleton (for review see Pienta and Coffey, 1984). Loops may exist in extended or more compact conformations (Igo-Kemenes *et al.*, 1982). Interest in such a model stems from proposals that at least some of these domains might be related to units of replication or transcription (Jackson *et al.*, 1984). These proposals are substantiated by the demonstrations that a variety of functional components, including steroid-dependent transcribing genes (Ciejek *et al.*, 1983 ; Jost and Seldran, 1984), newly synthesized and processed RNA (Herman *et al.*, 1978), as well as sites of DNA replication (Pardoll *et al.*, 1980 ; Tubo *et al.*, 1985), are closely associated with the nuclear matrix.

## B. — Characterization of active chromatin.

One obvious problem is how to explain the spatial architecture of active genes in relation to compacted inactive chromatin. Early observations pointed out that transcriptional activity was related to the chromatin decondensation (Pays and Flamand, 1976 ; Gottesfeld, 1977). Gene activation seemed to be linked with a conformational local relaxation in tightly-packed « inactive » chromatin. In agreement with these observations, nucleases appeared, as a rule, able to recognize some features of chromatin organization and degrade active genes more rapidly (Weintraub and Groudine, 1976 ; Levy and Dixon, 1978 ; Dimitriadis and Tata, 1980). The situation with respect to DNase I is in particular, most interesting.

DNase I sensitivity extends far upstream and downstream from the coding region for a gene (Stalder *et al.*, 1980a, b ; Bellard *et al.*, 1980 ; Lawson *et al.*, 1980 ; Storb *et al.*, 1981). In addition, this nuclease does not simply distinguish actively transcribing genes, but also those genes which have been transcribed, or will be transcribed during some later stage of development (see review : Weisbrod, 1982). « Active genes », defined by their sensitivity to DNase I, can thus be envisaged as lying in chromatin subunits or domains of « open » configuration, which seem to reflect more a potential for transcription than merely transcriptional activity (Mathis *et al.*, 1980 ; Lawson *et al.*, 1980 ; Stalder *et al.*, 1980a, b).

Digestion of chromatin by DNase I under very mild condition allows also the characterization of hypersensitive sites at specific positions relative to the coding region of genes (Wu, 1980 ; Groudine and Weintraub, 1981 ; Weintraub *et al.*, 1981). The precise structural basis of DNase I hypersensitivity is still the subject of considerable debate, but there is growing evidence that at least some hypersensitive sites are related to sequences involved in regulating gene expression (Dean *et al.*, 1983 ; Kaye *et al.*, 1984 ; Fritton *et al.*, 1984). Moreover, modulation of transcription may be governed by the binding of regulatory proteins to such hypersensitive regions (Emerson and Felsenfeld, 1984 ; Wu, 1984a, b). In short, it appears that the precise macrostructural organization (or disorganization) of chromatin determines which genes are (potentially) active. This might be governed, for instance, by cell differentiation. Gene expression itself would

require additional alterations of chromatin components, and/or interaction of regulatory factors with enhancer-like hypersensitive DNA sequences.

The molecular features which distinguish active from inactive chromatin have been the subject of numerous reports (Mathis *et al.*, 1980 ; Igo-Kemenes *et al.*, 1982 ; Weisbrod, 1982). They encompass post-synthetic modifications of DNA, histones and non-histone proteins. Yet, no single general molecular mechanism seems sufficient to account totally for hypersensitivity or transcriptional activity. As regards steroid hormones, there has been much emphasis on the presence of DNA recognition sites for high-affinity receptors, upstream from steroid-controlled genes. However, other observations also consider steroid hormones as potential modulators of chromatin macrostructure.

### C. — DNA signals for transcription.

The primary structure of eukaryotic DNA reveals important characteristics likely to be essential for an accurate and selective expression. Eukaryotic protein-coding genes are known to be split : the sequences (exons) coding for mRNA are interrupted by « non-coding » intervening sequences (or introns, IVS) and the entire split gene is transcribed into a precursor RNA (Abelson, 1979). The split gene phenomenon also applies to rRNA genes (Glover, 1983) and to tRNA genes (Clarkson, 1983 ; Peebles *et al.*, 1983 ; Greer *et al.*, 1983). In a protein-coding gene, each exon can be closely related to a functional or structural domain of the protein. Exons also appear well conserved through evolution. In contrast, introns have evolved rapidly, but can represent a major proportion of a gene (for review see : Breathnach and Chambon, 1981). Retention of the split-gene phenomenon may endow eukaryotes with selective advantages. By virtue of their IVS, eukaryotic genes might in theory have undergone many rearrangements throughout evolution.

A prerequisite for gene expression is the transcription (5'-3') of one DNA strand into a complementary RNA sequence. Distinct features allow DNA to be transcribed by the three different RNA polymerases. The nucleolar transcription of rRNA by RNA polymerase A (or I) involves a promoter lying between 320 nucleotides upstream and 113 nucleotides downstream from the DNA initiation site (Bakken *et al.*, 1982 ; Grummt, 1982). Termination of rRNA transcription apparently requires a cluster of at least 3 T residues at the 3' end of the transcription unit (Bakken *et al.*, 1982). RNA polymerase C (or III) transcribes genes coding for tRNA, 5S RNA and other small RNA (7S RNA, 7-3 RNA, La 4.5 RNA and Y RNA) (Busch *et al.*, 1982). Surprisingly, it appears that promoters for tRNA and 5S RNA genes are located within the genes themselves (Clarkson, 1983 ; Miller, 1983). RNA polymerase B (or II) transcribes those genes which code for mRNA as well as all capped small nuclear RNA, *e.g.* snU<sub>1</sub> to snU<sub>6</sub> (Busch *et al.*, 1982). More information is available about RNA polymerase B-dependent transcription, and the subject has been extensively reviewed (Abelson, 1979 ; Breathnach and Chambon, 1981 ; Nevins, 1983).

At least 2 regions have been delineated which are involved in initiation by RNA polymerase B. (1) The « TATA box » ( $\text{TATA} \begin{matrix} \text{A} \\ \text{T} \end{matrix} \text{A} \begin{matrix} \text{A} \\ \text{T} \end{matrix}$ ) is located 25-35 nucleotides upstream from the start site. This sequence seems to be involved in accurate positioning of RNA polymerase B molecules at the initiation site (Grosschedl and Birnstiel, 1980). (2) The « CAAT box » ( $\text{GC} \begin{matrix} \text{C} \\ \text{T} \end{matrix} \text{CAATCT}$ ), which is located about 70-80 base pairs (bp) upstream from the initiation site, appears to modulate mRNA transcription (Grosschedl and Birnstiel, 1980). Deletion of these promoters does not however eliminate transcription and it has not yet been demonstrated that RNA polymerase B binds to any of these sites. It seems that other sequences (enhancers), located far upstream from the actual start site, are also important for the initiation of transcription.

Although it is well established that initiation of transcription by RNA polymerase B occurs at the nucleotide corresponding to the 5' end (cap site) of RNA (Breathnach and Chambon, 1981 ; Nevins, 1983), the sequence(s) specifying termination of transcription by RNA polymerase B and the mechanism by which the RNA chain is released, remain unclear. A recognition signal (AATAAA, located 10-30 bp upstream from the 3' end) has been suggested to control RNA 3' end polyadenylation (Proudfoot and Brownlee, 1976). However, transcription often terminates beyond the site of poly(A) addition and the RNA 3' end seems in fact to be generated by RNA endonucleolytic cleavage rather than by real transcriptional termination. Multiple poly(A) sites are known to occur in « complex transcription units » (Amara *et al.*, 1982), and partial read-through across these sites can allow transcription of downstream exons. Such selection of a poly(A) site, and therefore termination of transcription, is obviously one control mechanism of gene expression (Rozek and Davidson, 1983 ; Nevins, 1983).

#### D. — Interaction of steroid-receptors with enhancer-like DNA sequences.

DNA sequences (enhancers), which confer upon particular genes their sensitivity to inducers, tend to be located in the 5'-flanking region. Amongst the many regulators of gene expression, transformed steroid-receptor complexes (RE\*) have been implicated in the control of transcription of steroid-dependent genes (Payvar *et al.*, 1981 ; Govindan *et al.*, 1982 ; Pfahl, 1982 ; Taylor and Smith, 1982b, 1985). DNA sequences that preferentially bind RE\* were also shown to exist in regions upstream from the transcriptional start site for genes controlled by progesterone (Mulvihill *et al.*, 1982 ; Compton *et al.*, 1983), glucocorticoids (Karin *et al.*, 1984 ; Scheidereit and Beato, 1984 ; Groner *et al.*, 1984), oestrogens (Jost *et al.*, 1984) and androgens (Davies, personal communication). No preferential binding site for RE\* has yet been demonstrated in genes other than those which code for proteins. The biological role of receptor binding to DNA recognition sites is now clearly established. Hybrid genes were constructed and used to transfect target cells known to contain specific receptors for steroid. These gene transfer experiments indicate that the promoter region of a steroid-controlled gene, which also contains DNA binding site(s) for the steroid-receptor complex, can be sufficient to confer hormone inducibility on an heterologous gene to which it is

linked in cis (Lee *et al.*, 1981 ; Renkawitz *et al.*, 1982 ; Dean *et al.*, 1983). According to these observations, steroid-receptors would therefore control steroid-dependent gene by interacting with enhancer-like DNA sequences. The DNA binding sites can also be located far upstream from the initiation site (Cato *et al.*, 1984), or even within introns of the transcription unit (Payvar *et al.*, 1981 ; Moore *et al.*, 1985). It has thus been conjectured that multiple receptor binding events might be required to alter chromatin structure across the entire transcription unit and increase transcription rates (Cato *et al.*, 1984). Nevertheless, the interaction of steroid-receptor complexes with enhancer-like DNA regions does not seem always sufficient to confer hormone inducibility.

Recognition sites also exist in genes not regulated by steroid hormones, and, more importantly do not exist in other genes which are regulated by steroids. No DNA binding site for dexamethasone-receptor seems to exist in glucocorticoid-dependent genes, such as rat growth hormone, rat uteroglobin or human proopiomelanocortin genes (see Moore *et al.*, 1985 ; Perry and Béchet, unpublished data).

In chick oviduct, two types (A and B) of high affinity receptors exist for progesterone and both types of progesterone-receptor complex (Prog-receptor) translocate to nuclei (Schrader and O'Malley, 1978). DNA recognition sites were described upstream from progesterone-controlled genes, but only for prog-receptor A. Prog-receptor B, in contrast, do not specifically interact with DNA, but preferentially bind to chromatin « acceptor sites » (Birnbaumer *et al.*, 1981). The exact nature of the nuclear acceptor sites for Prog-receptor B is still an area of extensive investigation (Spelsberg *et al.*, 1983). However, it would appear that progesterone-specific gene activity in chick oviduct is more closely correlated to the presence in nuclei of functional receptors B than to the existence of nuclear receptors A (Boyd-Leinen *et al.*, 1984).

Besides specific binding of steroids to high-affinity (Type I) receptors, there is additional evidence that cytosol and nuclei from various tissues contain other lower-affinity (Type II) binding sites for steroid hormones. In one instance, the importance of nuclear Type II sites in controlling rat uterine growth has been emphasized (Markaverich *et al.*, 1981 ; Clark and Markaverich, 1981). Although the exact nature of these Type II binding sites is not known, it is interesting to note that their nuclear acceptor sites do not seem to be related to DNA (Clark and Markaverich, 1982 ; Simmen *et al.*, 1984).

Therefore, in addition to steroid-receptors (Type I) interacting with enhancer-like DNA sequences, modulation of gene expression might also result from a steroidal regulation of chromatin macrostructure. This could involve, not only classical receptors, but also other low-affinity Type II steroid binding sites. In fact, any modification of DNA, or chromatin protein could alter chromatin organization and modulate transcriptional activity.

## E. — Steroidal control of chromatin organization.

### 1. — DNA methylation.

The most common modification of eukaryotic DNA is cytosine methylation, predominantly in the sequence CpG, which occurs in opposite pairs in the DNA duplex (Doerfler, 1983). Interest in DNA methylation has arisen partly from its ability to be perpetuated in a cell population. 5-Methylcytosine is inherited in a semiconservative fashion during replication, with newly synthesized DNA being accurately methylated early post-replication (Burdon and Adams, 1969) by maintenance DNA methylase(s) (Adams *et al.*, 1979). Nevertheless, the pattern of DNA methylation also evolves during tissue differentiation. Both *de novo* methylation of satellite DNA, as well as demethylation of specific active genes may occur during the course of development (Weintraub *et al.*, 1981). Though demethylation could simply result from an inhibition of maintenance DNA methylase, the identification of separate demethylating activities (Gjerset and Martin, 1982) and of *de novo* DNA methylases (Sano and Sager, 1980 ; Adams *et al.*, 1979) emphasize more the possible scope for modulators of gene expression in control of DNA methylation/demethylation. Indeed, in some cases, a strong relationship is seen to exist between undermethylation, tissue specificity, DNase I sensitivity and transcriptional activity (Weintraub *et al.*, 1981 ; Bird *et al.*, 1981 ; Naveh-Manly and Cedar, 1981). More precisely, the function of DNA methylation would depend mainly on a specific localization within or in the vicinity of regulatory sequences (Wilks *et al.*, 1982 ; Buslinger *et al.*, 1983 ; see also Doerfler, 1983).

One could argue that methylation (or demethylation) simply results from gene repression (or expression) on its own, and so is not regulatory. However two complementary mechanisms might account for a control of chromatin structure by DNA-methylation. First, double-stranded DNA (dsDNA) can assume different conformations, according to its environment, or as a result of specific DNA sequences. The classical B form is stabilized by nucleosome particles. Methylation, however, seems to stabilize Z-DNA (Behe and Felsenfeld, 1981). Demethylation of this latter conformation might impose a torsional stress on the DNA duplex and result in some unwinding of the double helix. Such regions would then be potential sites for replication or transcription (Nordheim *et al.*, 1981 ; Nordheim and Rich, 1983). Second, DNA methylation can alter DNA-protein interactions, and thereby directly control chromatin conformation and expression. The dependence upon either methylated or unmethylated DNA for specific restriction endonucleases to act (HpaII and MspI) exemplifies this point.

Relevant to these observations are the demonstrations that, *in vitro*, steroid-receptors are capable of protecting their DNA binding sites against methylation with dimethyl sulphate (Scheidereit and Beato, 1984 ; Karin *et al.*, 1984). Methylation of the DNA site can also prevent binding of the receptor (Cato *et al.*, 1984). *In vivo*, there is only limited information on whether the steroid-regulated demethylation of DNA results from or induces gene activity. In chicken liver,

oestradiol controls the transcription of Vitellogenin II gene and also brings out a precise demethylation of the oestradiol-receptor binding regions upstream from liver Vitellogenin II gene (Wilks *et al.*, 1982). In this case, demethylation occurs long after oestradiol induction of liver Vitellogenin and, therefore, seems only to result from transcriptional activity.

## 2. — *Alteration of nucleosome structure.*

It would be a very simple concept to imagine absence of nucleosome core particles as sufficient for transcriptional activity to proceed. Immunological (Scheer *et al.*, 1979) and nuclease digestion studies (Garel and Axel, 1976) have confirmed the presence of core histones in transcribing gene regions. The lesser compaction of active chromatin, rather than core histone depletion, might therefore be a better explanation of unfolding of the polynucleosome filament. The presence of histone isoforms and/or postsynthetic modifications of histones could release constraints upon DNA strands, and thereby alter nucleosome structure and the conformation of chromatin.

Histone variants, differing by just a few amino acids from the classical histones are known to occur (Von Holt *et al.*, 1979 ; Allis *et al.*, 1982). They provide some evidence for species-, tissue-, and gene- specific characteristics (Benezra *et al.*, 1981 ; Wu *et al.*, 1982a). A role in cell differentiation (Von Holt *et al.*, 1979) has been suggested by the occurrence of precisely-timed changes in histone subtypes during specific stages of development (Wu *et al.*, 1982b). Interestingly, the glucocorticoid-induced synthesis of mouse mammary tumor virus RNA in GR cells is highly correlated with changes in the relative amount of H1 variants (Wurtz, 1985). Whether these modifications result from a direct control by receptor-like molecules is unknown at the moment, but they suggest that, in this case, by changing the pattern of histone variants, steroid hormones might have the potential to induce rearrangements in chromatin structure.

Of the numerous post synthetic modifications histones can undergo (phosphorylation, acetylation, methylation, poly(ADP) ribosylation,...), histone acetylation seems to particularly characterize actively transcribing chromatin (Vidali *et al.*, 1978 ; Levy-Wilson *et al.*, 1979a ; Malik *et al.*, 1984). Acetylation of Lys residues occurs in all core histones, within the basic NH<sub>2</sub>-terminal region of the molecules, which also acts as the DNA-binding domain. Core histone acetylation might reduce their electrostatic interaction with DNA and so enhance DNA-template accessibility to RNA polymerase (Allfrey, 1982). Control of histone acetylation by steroid hormones has been referred to for oestradiol in target tissues, such as uterus (Libby, 1972 ; Pasqualini *et al.*, 1981) or liver (Pasqualini *et al.*, 1981), as well as for cortisol in rat liver (Graaf and Von Holt, 1973). Moreover, steroid-induced acetylation of histones is a very dynamic process (10 min ; Pasqualini *et al.*, 1981) which is well suited to a rapid regulation of gene expression.

Steroid receptors seem also capable of binding to core histone proteins. Kallos *et al.* (1981) have demonstrated, *in vitro*, preferential interactions between

transformed oestradiol-receptor complex and histones H2A and H2B. More data are obviously needed to clarify whether *in vivo* such phenomenons are relevant to the mode of action of steroid hormones. All these observation might, however, relate to processes whereby steroid-receptors could alter gene expression by means of controlled modifications of nucleosome conformation.

### 3. — *Involvement of non-histone proteins.*

Histones are certainly the best characterized DNA-binding proteins in eukaryotes, and their role as « packaging » proteins or non-specific repressors of gene expression is well established. SDS-polyacrylamide gel electrophoresis of « chromosomal proteins » also reveals an intricate and complicated pattern of non-histone proteins (NHP). High mobility group (HMG) proteins are NHP which have been extensively purified, characterized, and in some instances, specified as regulators of gene expression. HMG 1 (or 2) can unwind double-stranded DNA (Javaherian *et al.*, 1978), probably as a result of their selective affinity for single-stranded DNA (Isackson *et al.*, 1979). Such helix destabilizing properties have led to suggest possible involvement in DNA replication (Alexandrova *et al.*, 1984) or transcription (Goodwin and Mathew, 1982).

Several studies have associated HMG 14 (or 17) with actively transcribing chromatin (Weisbrod and Weintraub, 1979 ; Levy-Wilson *et al.*, 1979b). HMG 14 (17) seem able to recognize some structural characteristic(s) of chicken erythrocyte chromatin and, on binding to the region, induce DNase I sensitivity (Weisbrod and Weintraub, 1979 ; Gazit *et al.*, 1980). HMG 14 (17) are themselves subject to post-transcriptional modifications, such as acetylation-deacetylation or poly(ADP) ribosylation (Allfrey, 1982).

Interestingly, there is substantial evidence (Pasqualini *et al.*, 1981 ; Allfrey, personal communication) that oestradiol administration can induce acetylation of HMG proteins in target tissues. Moreover, there are also indications that the glucocorticoid-induced RNA synthesis in mouse mammary tumor cells is concomitant with poly(ADP) ribosylation of HMG 14 (17) proteins (Tanuma *et al.*, 1983). A control by steroid hormones of acetylation or poly(ADP) ribosylation of NHP such as HMG proteins could potentially result in modifications in chromatin conformation and gene accessibility to RNA polymerase.

Whether high-affinity receptors or low-affinity binding sites directly control post-synthetic modifications of DNA, histones, non-histone proteins or other constituents of chromatin remains unknown at the moment. Chromatin acceptor sites, other than DNA alone, have nevertheless been the subject of numerous reports (Perry and Lopez, 1978 ; Spelsberg and Halberg, 1980 ; Kon and Spelsberg, 1982 ; Ross and Ruh, 1984). The exact nature of the « acceptor proteins », together with the mechanism by which they regulate expression of specific genes is still under extensive investigation (see Spelsberg *et al.*, 1983). However, the acceptor proteins appear to exhibit tissue-specificity and to generate functional acceptor sites for steroid receptors only when bound to specific DNA sequences (Spelsberg *et al.*, 1984 ; Toyoda *et al.*, 1985). These observations would tend to suggest important functions in the control of gene

expression, despite the fact that no enzymatic activity has yet been associated with acceptor proteins.

### **Steroid control of the processing of the transcript**

DNA-dependent transcription results in the synthesis of pre-RNA molecules comprising both exon and intron transcripts. These large precursors (hnRNA) must undergo several obligatory processing events, in order to generate mature RNA molecules. All post-transcriptional processes of pre-RNA are potential sites for primary regulation of genetic expression. They govern accurate RNA capping, polyadenylation, splicing and stabilization. Thus, they determine which transcript will be transported to the cytoplasm for eventual translation. Essential requirements for adequate RNA processing events are both specific signals in the RNA nucleotides sequence, and appropriate enzymatic and « packaging » systems. After briefly summarizing RNA processing events, we will try to emphasize the limited but, we believe, significant data which suggest that steroid hormones can also control gene expression via a modulation of RNA processing.

#### **A. — RNA processing.**

##### **1. — Capping.**

The formation of a 5'-cap structure is coupled to initiation of transcription by RNA polymerase II (Jove and Manley, 1982). The cap structure might be involved in protection of RNA against nucleolytic attack as well as be involved in RNA splicing events (Nevins, 1983).

##### **2. — Polyadenylation.**

Poly(A) addition to the pre-mRNA 3'end occurs 11-19 nucleotides downstream from the consensus sequence AAUAAA. Recent reports (Gil and Proudfoot, 1984 ; see review by : Birnstiel *et al.*, 1985) suggest that this hexanucleotide together with additional sequences act as recognition sites for proper endonucleolytic cleavage of the nascent RNA chain. The new pre-mRNA 3'end, so formed, is then the site of polyadenylation. Poly(A) addition is a rapid process and occurs very early on the nascent pre-mRNA chain (Salditt-Georgieff *et al.*, 1980b ; Nevins, 1983). Poly(A) polymerase has been identified immunologically (Rose *et al.*, 1979) as the 75,000-Mr poly(A) binding protein (Roy *et al.*, 1979). Pre-mRNA polyadenylation might also be directed by hybridization of the nascent RNA with small nuclear RNA U<sub>4</sub> (U<sub>4</sub> snRNA) (Berget, 1984) and/or U1 snRNA (Moore and Sharp, 1984). Other, yet unknown, components of the

polyadenylation machinery might also be involved, in order to select the correct poly(A) addition site in complex transcriptional units (Nevins, 1983). There is evidence that the poly(A) tail determines the stability of RNA transcripts (Huez *et al.*, 1981) and particularly of mRNA in cytoplasm (Zeevi *et al.*, 1982).

### 3. — *Splicing.*

The splicing process ensures both excision of intron transcripts from the pre-RNA chain and accurate ligation of exon transcripts. Individual intron transcripts are excised from pre-RNA in several steps which have recently been described for protein-coding RNA (Konarska *et al.*, 1985 ; Reed and Maniatis, 1985). First, the 5'-splice site is cleaved and the 5'-end of the intron (a G-residue) forms a phosphodiester bond to a A-residue inside the same intron. This branch point is located 20-40 nucleotides upstream from the 3'-splice site. The second step involves the excision of the intron as a lariat form and the concomitant ligation of the two exons.

A part from involvement of RNA primary sequences, accuracy of splicing for the most part depends also on hnRNA-interactions with other RNA and specific proteins. Among RNA molecules which have been proposed to guide the splicing events are small nuclear RNA's (snRNA). Some exist hydrogen bonded to hnRNA (Gallinaro and Jacob, 1981 ; Zieve and Penman, 1981 ; Serekis and Guialis, 1981 ; Setyono and Pederson, 1984). Moreover, anti-snRNP antibodies have been demonstrated to inhibit hnRNA splicing (Yang *et al.*, 1981). U<sub>1</sub> snRNA, especially, exhibits a 5'-sequence strikingly complementary to the splice junction (Lerner *et al.*, 1980 ; Rogers and Wall, 1980). These observations have led to the proposal that U<sub>1</sub> snRNA might hybridize to pre-mRNA and be involved in splicing of hnRNA (Gallinaro *et al.*, 1981 ; Busch *et al.*, 1982 ; Di Maria *et al.*, 1985).

HnRNA and snRNA also exist *in vivo* as ribonucleoprotein particles (hnRNP and snRNP, respectively). It is thus important to consider that splicing must occur within highly organized ribonucleoprotein multicomponents, somewhat analogous to ribosomes (Brody and Abelson, 1985 ; Grabowski *et al.*, 1985 ; Frendewey and Keller, 1985).

### 4. — *RNA stability.*

Even with adequate mechanisms for RNA transcription, capping, polyadenylation or splicing, the delivery of mature RNA from nucleus into cytoplasm can also be affected by the relative stabilities of pre-, intermediate- or mature-RNA. In addition, expression of a particular gene will be more efficiently switched off by simultaneous repression of transcription with controlled degradation of pre-existing RNA. RNA processing events, such as 5'-capping (Nevins, 1983) and poly(A) addition (Huez *et al.*, 1981) have been suggested as protecting RNA against nucleolytic degradation.

## 5. — Nucleocytoplasmic transport of RNA.

Mature RNA is then transported into cytoplasm through the nuclear pore complex (for a recent review see Clawson *et al.*, 1985). The precise mechanism of transport remains unknown, but it does exhibit selectivity towards correctly processed mRNA (Webb *et al.*, 1981) or rRNA (Wunderlich, 1981). Accurate splicing of pre-RNA to mature RNA is apparently a prerequisite for nucleocytoplasmic transport. RNA transport is also an energy-dependent process and involves a nucleoside triphosphatase associated with nuclear envelope and matrix (Clawson *et al.*, 1985).

## B. — Ribonucleoprotein complexes and higher order structures.

Nuclear RNA co-exists with specific proteins in highly complex macrostructures (hnRNP), whose architecture is somehow controlled by the nuclear skeleton (matrix). A simplified scheme is to imagine nascent RNA extending from transcriptionally active chromatin, itself looped-out from condensed heterochromatin (Sommerville, 1981 ; Vlad, 1983). Nascent transcripts are attached to the DNP axis by RNA polymerase molecules, and as transcription proceeds, newly-formed RNA arise as a gradient of fibrils of increasing length (Franke and Scheer, 1978 ; Puvion and Moyne, 1981). Specific proteins rapidly bind to nascent RNA immediately adjacent to RNA polymerase molecules (Sommerville, 1981). hnRNP fibrils are commonly observed as « 20-30 nm beads on a string », somewhat analogous to nucleosomal DNP fibrils, and a major set of closely-related polypeptides is considered to generate and maintain this packaging of hnRNP (Leser *et al.*, 1984 ; Choi and Dreyfuss, 1984 ; Wilk *et al.*, 1985). Likewise, preribosomal structures are evident before transcription of rRNA precursor is completed (Glover, 1983).

Close observations indicate, however, other diverse configurations for nascent RNA, even along the length of a single transcript (Sommerville, 1981). Thus, superimposed on the simple « ribonucleosomal » model, more complex structures exist. In addition to specific protein-protein or protein-RNA interactions, RNA base-pairing can occur within the same molecule (Jelinek and Darnell, 1972 ; Jelinek *et al.*, 1974 ; Kish and Pederson, 1977) or with other RNA (Brunel *et al.*, 1981 ; Gallinaro *et al.*, 1981 ; Setyono and Pederson, 1984). All these highly organized configurations of RNP might be expected to influence processing events. Certain snRNP seem to play a central role in splicing and poly(A) addition, if not most RNA processing events. The nuclear skeleton appears to support DNA replication (Pardoll *et al.*, 1980 ; Tubo *et al.*, 1985) and transcription (Robinson *et al.*, 1983 ; Ciejek *et al.*, 1983 ; Jost and Seldran, 1984) by tightly anchoring DNP. This structure also binds hnRNP (Herman *et al.*, 1978 ; Miller *et al.*, 1978a ; Van Eekelen and Van Venrooij, 1981) and snRNP (Miller *et al.*, 1978b ; Gallinaro *et al.*, 1983), as if it is equally involved in RNA processing events. Concerted transport and processing of nascent RNP to mature-RNP thus

resemble an « assembly line », from the DNP transcriptional unit to the nuclear pore complex, where the role of the « conveyor belt » might be played by the nuclear matrix.

### C. — RNP processing and steroid hormones.

*In vitro*, cytosolic steroid-receptor complexes not only bind to DNA, but also demonstrate significant interactions with RNA (Economidis and Rousseau, 1985). RNA is a potent competitor for the binding of receptor- androgen (Liao *et al.*, 1980), -oestrogen (Feldman *et al.*, 1981 ; Chong and Lippman, 1982), and -dexamethasone (Tymoczko *et al.*, 1982) complexes to DNA-cellulose. Moreover, rRNA, tRNA and poly(A) RNA, all are capable of promoting release of receptor complexes that were bound to DNA *in vitro* (Liao *et al.*, 1980). There is also some evidence that steroid-receptor complexes can interact *in vitro* with ribonucleoprotein particles isolated from uterine cytosol (Liang and Liao, 1974), as well as from prostate and uterine nuclei (Liao *et al.*, 1973).

Selective recognition of RNA or RNP by steroid-receptor complexes might suggest a post-transcriptional role in RNA processing. Regulation of genetic expression by steroid hormones may not simply be due only to an interaction of receptor complexes with DNA regulatory regions or to modulation of the conformation of the DNP axis. RNA might compete with DNA for the polynucleotide binding site of the steroid receptor. A preferential binding of nuclear chicken oviduct oestrogen-receptor to poly(A) RNA was suggested by Lin and Ohno (1983). Such RNA-receptor interactions might be relevant to the reported stabilization of specific mRNA by steroid hormones. The half-life of ovalbumin mRNA was significantly reduced in oestrogen withdrawn chick oviduct (Palmiter and Carey, 1974 ; Cox, 1977) ; similarly, oestrogen or progesterone was demonstrated to affect the half-life of colnalbumin mRNA in chick oviduct (Mc Knight and Palmiter, 1979), and androgen to modulate the half-life of prostatic binding protein mRNAs (Page and Parker, 1982).

Many other roles for steroid-receptor complexes can be envisaged in RNA processing or transport. Direct evidences supporting the concept that oestradiol stimulates the nucleocytoplasmic transport of RNP in rat uterine nuclei were presented by Vazquez-Nin *et al.*, (1978, 1979) and more recently by Thampan (1985). Furthermore, most interesting is that, when nuclear matrix fulfills the structural requirement for a conveyor belt for RNP processing and transport, oestrogen and androgen receptors have also been considered integral components of this skeleton (Barrack and Coffey, 1980 ; Béchet *et al.*, 1986b). In this context, receptor-RNA interaction might not only be an important mediator of RNA processing and transport, but also a component of receptor processing and/or transport back to the « cytosol ». Recycling of nuclear steroid-receptors to their cytosolic form nevertheless remains an enigma (Horwitz and Mc Guire, 1980 ; Kasid *et al.*, 1984), yet there is evidence that untransformed « cytosolic receptors » can exist complexed with RNA (Chong and Lippman, 1982 ; Tymoczko and Phillips, 1983 ; Economidis and Rousseau, 1985).

## Conclusions

Despite extensive scientific interest in steroids and anabolic agents, the exact mechanism(s) of action for these hormones at the sub-cellular level remain(s) to be elucidated. Steroid-receptor complex formation requires preliminary « *activation* » of the receptor to a binding state, and « *translocation* » to nuclei involves transformation of the steroid-receptor complex to a nucleophilic form. Only recently have observations begun to clarify the molecular modifications and/or interactions with other factors that occur when receptors undergo the activation and transformation processes. In this development even basic principles, such as the « two step hypothesis », have become suspected as inaccurate, with the precise cellular location of untransformed steroid receptors in controversy.

Steroids are capable of modulating gene expression, and recently there has been considerable emphasis placed on the recognition, by transformed steroid-receptors, of specific DNA sequences upstream from steroid controlled genes. The postulate is that steroids modulate transcription by interacting, via high-affinity receptors, with enhancer-like DNA regions. However, the only recognition by steroid-receptors of enhancer-like DNA sequences do not explain why the same steroid receptor do not regulate the same gene within different target tissues. If we exclude a tissue-specific rearrangement of DNA control regions during differentiation, the primary structure of DNA is therefore insufficient to totally account for a transcriptional control by hormone-receptor complexes. In fact, eukaryotic DNA does not execute its functions as an isolated simple unit. Transcription requires decondensation of a highly organized complex of DNA with histones, non-histone and scaffold proteins. Many alterations of this macrostructure are possible which might result in enhanced DNA-template accessibility to RNA polymerases. Such additional codes superimposed on DNA primary structures are likely to determine which genes are (potentially) active. Substantial evidence also indicates that steroids might efficiently control gene expression via such modification in chromatin conformation.

In addition to DNA transcription, many other processes are also available as potential mechanisms of control over gene expression. Proper maturation and transport of pre-RNP to mature-RNP is essential. Steroid hormones have been shown to affect the stability of specific RNA, and to modulate RNA nucleocytoplasmic transport, if not other RNA processing events.

An alternative view to steroid-receptors acting as a specific key to a single lock might therefore be to consider steroid binders as capable of modulating different aspects of gene expression, from DNA transcription to RNA transport by acting as a « master » key to several locks. By this, a range of control mechanisms might exist for a particular steroid to modulate the expression of different genes, and in different tissues this « mix » of control could be variable.

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**Résumé.** *Contrôle de l'expression génétique par les hormones stéroïdes.*

Le mécanisme d'action des hormones stéroïdes implique leur interaction avec des sites de liaison spécifiques du tissu cible, de laquelle résulte une modulation précise de l'expression génétique. Dans les tissus cibles, il existe pour les hormones stéroïdes des récepteurs à haute affinité, ainsi que des sites de liaison secondaire. Dans plusieurs cas, il a été démontré que les complexes hormone-récepteur sont capables de réguler directement la transcription, ceci en se liant à des régions de l'ADN situées à proximité des gènes contrôlés. Cependant, d'autres données expérimentales suggèrent que les hormones stéroïdes pourraient aussi moduler la transcription en modifiant la structure de la chromatine. Dans ce cas, leur action se traduirait par des modifications post-traductionnelles de protéines histones et non-histones, ainsi que par des variations des proportions relatives des isoformes d'histones. Hormis la transcription, il est aussi désormais concevable que les hormones stéroïdes modulent effectivement l'expression génétique en régulant certaines étapes de la maturation des ARN. Le rôle respectif de récepteurs de haute affinité ou de sites secondaires dans un contrôle direct de ces phénomènes reste cependant inconnu. Ces quelques remarques suggèrent l'existence de plusieurs niveaux d'action permettant d'assurer un contrôle précis de l'expression génétique par les hormones stéroïdes.

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