科技资料

Endocrine Therapy of Breast Cancer IV

A. Goldhirsch (Ed.)

Endocrine Therapy of Breast Cancer IV

With 19 Figures and 40 Tables

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Foreword

The European School of Oncology came into existence to respond to a need for information, education and training in the field of the diagnosis and treatment of cancer. There are two main reasons why such an initiative was considered necessary. Firstly, the teaching of oncology requires a rigorously multidisciplinary approach which is difficult for the Universities to put into practice since their system is mainly disciplinary orientated. Secondly, the rate of technological development that impinges on the diagnosis and treatment of cancer has been so rapid that it is not an easy task for medical faculties to adapt their curricula flexibly.

With its residential courses for organ pathologies and the seminars on new techniques (laser, monoclonal antibodies, imaging techniques etc.) or on the principal therapeutic controversies (conservative or mutilating surgery, primary or adjuvant chemotherapy, radiotherapy alone or integrated), it is the ambition of the European School of Oncology to fill a cultural and scientific gap and, thereby, create a bridge between the University and Industry and between these two and daily medical practice.

One of the more recent initiatives of ESO has been the institution of permanent study groups, also called task forces, where a limited number of leading experts are invited to meet once a year with the aim of defining the state of the art and possibly reaching a consensus on future developments in specific fields of oncology.

The ESO Monograph series was designed with the specific purpose of disseminating the results of these study group meetings, and providing concise and updated reviews of the topic discussed.

It was decided to keep the layout relatively simple, in order to restrict the costs and make the monographs available in the shortest possible time, thus overcoming a common problem in medical literature: that of the material being outdated even before publication.

Umberto Veronesi
Chairman Scientific Committee
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Introduction

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This is the fourth issue of our Monograph on Endocrine Therapy of Breast Cancer. As in the past, this volume is the result of highly interesting discussions among the members of the Task Force and several guests, all of them outstanding researchers in their respective fields. To discuss controversial issues pertaining to data deriving from one's own work is an extremely pleasant exercise, and at the same time generates both sound criticism and new hypotheses; the latter is essential for the continuation of productive research. The 1990 edition contains the following four items of notable interest: 1) new data concerning the function of oestrogen and progesterone in promoting receptor-mediated growth; 2) a definition of prognostic factors in breast cancer, particularly in node-negative disease; 3) new data about "old" endocrine therapies; and 4) a discussion of adjuvant therapies and the measure of their benefit, with special emphasis on quality-of-life considerations.

Each of the chapters provides new data or discusses features of interest to individuals who are intellectually involved with breast cancer: Dr. King challenges the role of oestrogens in cell growth and differentiation by introducing new "actors", progesterone and progestins. New views regarding receptors and oestrogen are discussed by Dr. Milgrom. The prognosis of breast cancer is reviewed by Dr. Klijn, especially in relation to growth factors and their receptors, and by Dr. Mouridsen and other members of the Danish Breast Cancer Study Group, who report their findings about node-negative disease. In a section on endocrine therapeutics, Dr. Jordan provides new data on the long-term use of tamoxifen, Dr. Howell discusses endocrine mechanisms which should be reconsidered and re-examined, and Dr. Milsted reviews the status of LHRH-superanalogues. Adjuvant systemic therapies are also dealt with by Dr. Kaufmann in his review of new node-negative trials, and by Drs. Gelber, Castiglione and Goldhirsch, whose new data indicate that endocrine mechanisms are not solely responsible for the effect of adjuvant systemic chemotherapy in premenopausal patients. The methodological controversy about how best to define the benefit from a therapy which provides only modest treatment effects is extensively described by Dr. Gelber.

Endocrine mechanisms and breast cancer continue to be fascinating subjects for research which represent fertile areas for the germination of hypotheses. When the Task Force will meet and produce its fifth edition in 1991, new revelations are certain to have come to light which will serve both to nurture and reward our interest in this field.

Role of Oestrogen and Progestin in Human Mammary Carcinogenesis

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From an endocrinological aspect, the view that oestrogens are the major adverse factor in human breast cancer has dominated thinking in this area [1,2]. This opinion is based on three main lines of evidence; (a) the ability of oestrogens to generate mammary tumours in rodents [3,4]; (b) epidemiologically-derived risk factors such as the protective effect of ovariectomy and increased risk of breast cancer in young women given diethylstilboestrol to prevent abortion [1,2]; and (c) the mitogenic effects of oestrogens on established breast cancer cell lines [5,6] and efficacy of antioestrogens in treating established breast cancer [7].

Conversely, the other ovarian steroid progesterone and its synthetic derivatives (progestins) are thought to be protective, a view largely based on their antioestrogenic and therefore antiproliferative effects on endometrium [8]. Supportive evidence for beneficial effects of progestins comes from their clinical use in advanced breast cancer [9] and their ability to decrease tumour yield under certain conditions in rodents [3,4].

Many of the data on which the above model is based are capable of the alternative explanation that, as far as early stages of breast cancer induction are concerned, progestins are not good but bad and oestrogens may play a more permissive role. This has been termed the "oestrogen plus progestagen" hypothesis [10], which is mainly based on two types of observations. In contrast to endometrium, *in vivo* proliferation of normal human breast epithelium is maximal during the progestagenic phase of the cycle and the contraceptive pill stimulates proliferation [11-15] together with publications suggesting an increased risk of breast cancer in young women on the contraceptive pill [16,17] and with one report of a progestin-related breast cancer risk in women on hormone replacement therapy [18]. It must, however, be stressed that neither of these sets of epidemiological data should be considered proven.

Given the importance of deciding whether oestrogen alone or oestrogen plus progestin adversely affects human breast cancer, resolution of the question is imperative. Currently, insufficient data are available to achieve this objective. The purpose of this chapter is to highlight some of the more important points that need resolution.

Animal Studies

Oestrogens alone can induce mammary tumours in mice [3,4]; this could be used as evidence against a progestin involvement. However, a progestagenic environment increases tumour incidence [3,4], so progestins can be stimulatory. The endocrine requirements of hydrocarbon-induced mammary tumours are complex and vary according to species and whether the manipulations are carried out before or after hydrocarbon administration. Depending on conditions, progestins can either decrease or increase tumour development [3,4,19]. Thus, in relation to the human situation, the animal data are inconclusive in deciding between the two models.

Risk Factors

Ovariectomy clearly protects against subsequent development of breast cancer [1,2] but, as this operation removes both oestrogen and progestin, its interpretation is equivocal. Likewise, increased tumour incidence in women who received diethylstilboestrol for threatened abortion occurred against the progestagenic background of pregnancy [1,2]; increased progestin potency in that oestrogenic environment cannot be discounted. The increased risk due to obesity [1,2] could be explained in the same way for premenopausal women, but the postmenopausal situation would be more problematic.

In the original "bad oestrogen" hypothesis, it was thought that, with early menarche, the initial cycles were anovulatory and therefore progestin deficient [20], but this is now thought to be incorrect [21,22], so that early menarche establishes early exposure to progesterone. Thus, the "oestrogen alone" model is less compatible with the menarche data than the "oestrogen plus progestin" hypothesis.

Late menopause [1,2] does not immediately fit with the progestin model as such cycles tend to be anovular [22]; several explanations are possible. If hormonal sensitivity changes with progression (see below), it is possible that the breast cells at risk are different at the two extremes of reproductive life and that they should be considered as being at different stages of progression. Alternatively, one could argue, as others have done [22], that the total number of ovulatory cycles (oestrogen and progestin) is the important feature and that the late menopause reflects an increased number of such cycles, even though the last ones are anovular.

An early, first full-term pregnancy markedly decreases the risk of subsequent breast cancer, an effect that has been ascribed to the highly progestagenic milieu of pregnancy [1,2]. This could argue against a bad effect of progestins, but the hormonal environment of pregnancy is not the same as that of the luteal phase and this is reflected in the physiological response of the mammary lobules. In the normal cycle, the intense lobular development associated with pregnancy does not occur and epithelial dedifferentiation is less evident [19]. Pregnancy-related differentiation makes the epithelial cells more resistant to carcinogens [19], an effect that may not occur in the normal cycle. Intriguingly, pregnancy results in a long-term desensitisation to the proliferating effects of the contraceptive pill on breast epithelium [12].

Explaining the various risk factors by either model alone is difficult. More biological data are required about the various physiological situations that can be related to the rather heterogeneous collection of risk factors.

Mitogenic Effects of Oestrogens; Antioestrogenic Effects of Progestins

Cell proliferation is a vital component in carcinogenesis both at the level of increasing the number of target cells for initiating agents and in amplifying abnormal cell populations after initiation. Hence, oestrogen and progestin effects on proliferation are relevant to the topic of this chapter.

Most of the data on female sex steroids and cell proliferation have been generated from studies on normal endometrium and breast cancer cell lines. As there is a possibility that hormone sensitivity alters during progression (see below), effects on normal and cancer cells will be considered separately.

Normal Cells

There is no doubt that oestrogens are mitogens for endometrial cells and that progestins counteract that effect [8], but the relevance of those data to normal human breast epithelium is questionable. Several groups have demonstrated that normal breast lobular-alveolar epithelium exhibits greater proliferation in the luteal than follicular phase of the menstrual cycle [11-15]. This clear-cut difference to endometrium indicates that, if oestrogens stimulate breast epithelial proliferation, it is by a less direct route than with endometrium and progestins could be a component distal to oestrogen in the breast. The simplest explanation of the *in vivo* breast data is that progesterone is the proliferative agent, a view that is enhanced by the finding that the

contraceptive pill, in particular progestin-only pills, increases luteal phase proliferation [12]. At the very least, there are no data that progestins inhibit oestrogen-induced proliferation in normal, human breast epithelium *in vivo*. In cell culture the situation may be different (see below).

A proliferative effect of progestins could result either in an increased number of targets for initiating agents or change the susceptibility of the epithelial cells to those agents.

Oestrogens, by increasing progesterone receptor levels, are known to increase progestin potency. This could also occur with human mammary epithelium. Alternatively, oestrogens might have a direct mitogenic effect other than via progesterone receptor, although the *in vivo* data indicate that, if so, the effect is small in relation to that of progestins. There is a very low proliferation during the oestrogenic phase of the cycle [11-15], which could be due either to a basal activity or an oestrogenic influence. These *in vivo* data are at variance with cell-culture [23] and nude-mouse [24] results indicating that oestrogens are mitogenic for human mammary epithelium and progestins are inhibitory. The basis for these discrepant results should be urgently identified. Four independent groups have established that, *in vivo*, lobular epithelium proliferates faster during the luteal phase of the cycle [11-15], so this can be taken as proven. An indirect effect of progestins on mammary epithelium is one possible explanation of the discrepant behaviour in culture and *in vivo*, but would not explain the nude-mouse data without invoking species differences. This is an unlikely explanation as rodent mammary epithelium behaves like the human in proliferating out of phase with that of uterine epithelium [25].

Established Breast Cancer

Oestrogens are well established as being the main steroidal mitogens for established breast cancer [5-7] and may well promote preneoplastic lesions to a more malignant state. Given the menstrual cycle and pill data indicating a proliferative effect of progestins on normal epithelium (see above), it is possible that a change in sensitivity profile occurs at some state in the carcinogenic process.

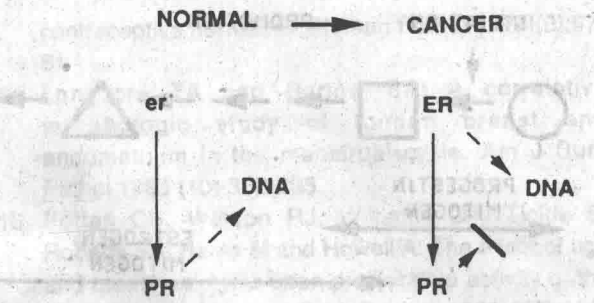

Fig. 1. Upregulation of oestrogen receptor (ER) as a potential mechanism for changing steroid sensitivity during human mammary carcinogenesis. In normal cells, low levels of oestrogen receptor (er) can upregulate progesterone receptor (PR) which in the presence of a progestin increases DNA synthesis. Possibly, er may have a small, direct effect on DNA (not shown). Upregulation of ER in cancer cells increases/changes their sensitivity to oestrogen; additional changes alter progestin responses. This block may not be complete (not shown)

This is known to occur in rat models, although in that situation progestin effects are the opposite [3,19] of those being hypothesised here for human mammary carcinogenesis. A possible mechanism for such a switch is mentioned below.

Effects of progestins on established breast cancer are poorly defined. Pharmacologic levels of progestins can induce regressions in advanced breast cancer [9], whilst physiological levels can inhibit growth of human breast cancer cell lines [26,27]. However, all of the latter experiments were performed in the oestrogenic environment of phenol red; recent data obtained in the absence of phenol red indicate that progestins can have a weak proliferative activity [28,29]. There are two conflicting reports [26,28] on the actions in cell culture of the antiprogestin RU486 in the absence of oestrogen.

Altered Steroid Sensitivity Due to Progression

The mitogenic effect of oestradiol on breast cancer cells is proven and there must therefore be a change in steroid sensitivity from

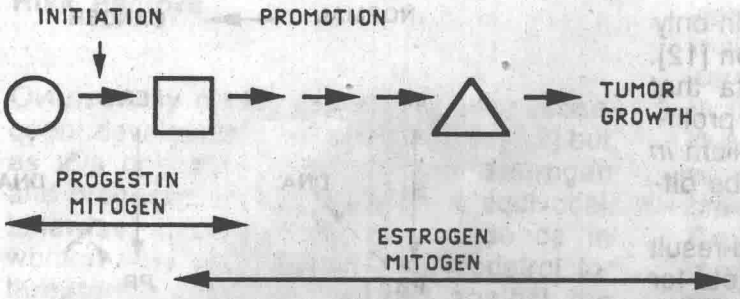

Fig. 2. A model of progestin and oestrogen involvement in human mammary carcinogenesis. An hypothetical switch in steroid sensitivity occurs during progression and is depicted here as occurring at an early stage of promotion; it could occur at a later stage. Other features of the model are listed in Table 1

progestin to oestrogen somewhere along the progression pathway. One candidate mechanism for such a change might be the upregulation of oestrogen receptor (ER) that occurs during progression [30,31] (Fig. 1). It is now clear from molecular biological studies that steroid sensitivity is markedly dependent on the number of receptors per cell [32,33]. Thus, the increased ER content of some breast tumours relative to that seen in normal mammary epithelium could result in heightened oestrogen sensitivity. This cannot be the only change, otherwise one would predict that progestins should be strongly mitogenic for the cancer cells, which is not the case. However, recent data with human breast cancer cell lines indicate that progestins can retain weak proliferative activity under certain conditions in such advanced breast cancer cells [28,29]. Thus, the effects of physiological levels of progestagens on breast cancer cell proliferation are unclear, although pharmacologic levels are undoubtedly cytotoxic [9].

Conclusions

Sufficient doubts exist to question the view that oestrogens alone adversely influence human mammary carcinogenesis particularly in its early stages. The alternative oestrogen plus progestagen view warrants more attention and one possible model is illustrated in Figure 2, with its main features listed in Table 1. Two essential differences from the oestrogen-alone model are that progestins are not benign or even beneficial agents and that the steroid effect varies with stage of neoplastic process. This model is compatible with the existing data outlined above.

None of the points and counterpoints made in this chapter lead to firm conclusions and more data are urgently required to establish the validity or otherwise of many of the arguments presented. The possibility of progestins having adverse effects on early stages of human breast carcinogenesis deserves further consideration as it has important consequences. The influence of the contraceptive pill is a case in point, but discussions on ways of preventing breast cancer are taking place; these are largely based on the oestrogen model [34,35,36]. These should continue, but additional thinking about anti-progestins is called for as they may have inherent advantages over antioestrogens, whilst the use of progestins for this purpose may be counterproductive. At the cell biological and biochemical level, oestrogens have dominated thinking and practical effort; progestins warrant at least equal attention.

Table 1. Main features of an oestrogen plus progestin model of human mammary carcinogenesis

1	Progestins, by their mitogenic effect, increase the probability of successful initiation/early promotion events
2	Oestrogens, by inducing progesterone receptor, increase the mitogenic potency of progestins
3	A change in steroid sensitivity accompanies progression so that oestrogens become mitogenic for established cancer cells and possibly for preneoplastic cells

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Molecular Genetics of Steroid Hormone Receptors

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Interest in steroid hormone receptors in breast cancer stems from both theoretical and practical considerations. The malignant transformation and subsequent growth of breast cancer cells are hormonally regulated, and elucidation of the mechanisms of these processes requires an understanding of the structure and function of hormonal receptors. Moreover, receptor determination in tumour biopsies has now been used for many years as a means of predicting response to hormonal therapy and as prognostic factors in early breast cancer. Recent cloning of most of these receptors has allowed researchers to obtain a considerable wealth of new information and has provided new tools with which further questions become amenable to experimental analysis (reviews in [1-3]).

Cloning and Sequencing Analysis of Steroid Hormone Receptors

Glucocorticoid [4] and oestrogen [5,6] receptors were the first to be cloned and sequenced, followed by progesterone receptors [7-10]. In all cases, prokaryotic expression vectors were used and receptor encoding clones were detected by the binding of antibodies. This breakthrough was thus dependent on the preparation of antibodies of adequate specificity and sensitivity for detection. At this stage, the similarity in the DNA binding domains of various receptors had been established and this led to isolation, by cross-hybridisation, of other receptors, including the aldosterone [11] and androgen receptors [12-15]. The thyroid hormone receptors were isolated as the normal cellular equivalents of the viral oncogene v-*erb*-A [16,17]. Sequencing showed these cDNAs to encode proteins of various length (Fig. 1) (595 amino acids for the oestrogen receptor, 933 amino acids for the progesterone receptor, 918 amino acids for the androgen receptor, etc). However, in all cases, the receptors could be aligned through a central Cysteine-rich basic amino-acid region, shown in subsequent experiments to be the DNA binding domain.

Comparison of the structure of a given receptor in several species allows one to define the functional domains of the receptor. For instance, in the case of the progesterone receptor, comparison between human, rabbit and chick receptors shows a 100% conservation of the DNA binding domain. This is a general feature of all the receptors and the total conservation of this domain (although in some cases changes of a single amino acid have been described) explains why receptors - regardless of the species of origin - have proven to be effective in DNA transfection experiments on target genes from different species.

The C-terminal part of the receptor constitutes the steroid binding domain and it is separated from the DNA binding domain by the so-called hinge region. It is also markedly conserved among mammalian species (between human and rabbit progesterone receptors, only one amino acid is different), but there exists some divergence from the avian receptor. This difference in amino acid sequence is mirrored by differences in steroid binding specificity. For instance, RU 486 binds to the mammalian receptor and antagonises the action of progesterone, whereas it

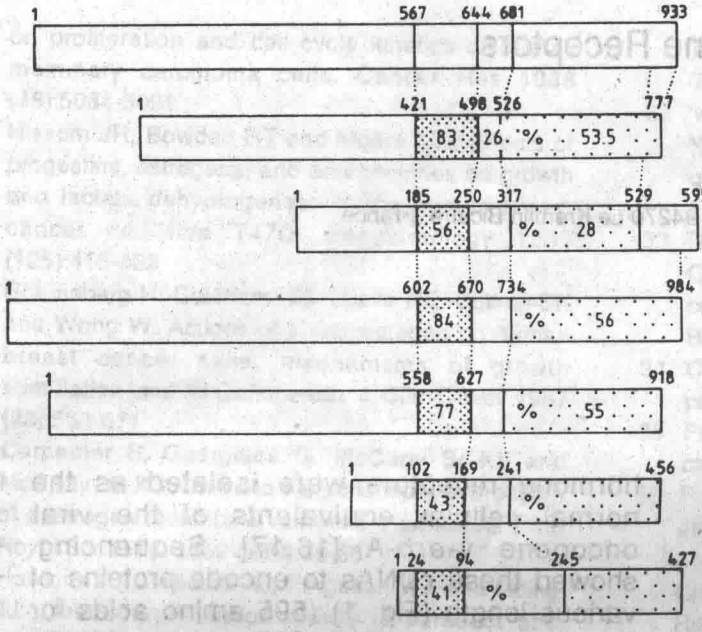

Fig. 1. Schematic comparison between human progesterone (hPR), glucocorticoid (hGR), oestrogen (hER), mineralocorticoid (hMR), androgen (hAR), thyroid (hc-erb-aß) and vitamin D (h VDR) receptors.
Receptors are aligned through their DNA binding domain (dark boxes). Steroid binding domains are shown by dotted boxes. Percent homology to progesterone receptor is indicated

does not bind to the chick receptor and is inactive as a progesterone antagonist in this species. The N-terminal half of the receptors is the most variable region, both in length and in amino-acid sequence. It contains some transcription modulating sequences, and it is found to be the major antigenic region when epitopes recognised by monoclonal antibodies against glucocorticoid and progesterone receptors are mapped [18].

Subfamilies Among Nuclear Receptors. Relationship with Oncogenes and Anti-Oncogenes

This family of proteins involves not only receptors for steroid hormones but also receptors from derivatives of lipophilic vitamins (vitamin D [19,20] and retinoic acid [21-25]) and thyroid hormones [16,17]. Various morphogenetic and developmental regulators [26-28] or transcription factors [29,30] with no known receptor function have also been described. The fact that these proteins may, especially when modified, play a role as oncogenes and anti-oncogenes, is best exemplified by the history of the discovery of the thyroid and retinoic acid receptors. Avian erythroblastosis virus contains 2 oncogenes: v-*erb*-B, which is a truncated derivative of the EGF receptor, and v-*erb*-A, whose function was unknown until the glucocorticoid receptor had been cloned and sequenced. It was then found by random computer search that the DNA binding domain of the receptor had a marked similarity to a region of v-*erb*-A [31]. It was thus suspected that the latter might be a viral derivative of a normal cellular gene having some receptor function. This observation led to isolation by cross-hybridisation of the cDNA encoding c-*erb*-A (the normal cellular equivalent of v-*erb*-A). It was subsequently established that c-*erb*-A bound triiodothyronine and was thus the thyroid hormone receptor [16,17]. Several variants of this receptor were later identified and shown to be variably expressed in different tissues [2,32-34]. v-*erb*-A was found to be a non-ligand binding equivalent of c-*erb*-A and to exert an inhibitory action on its biological activity. v-*erb*-A bound to thyroid hormone-responsive elements without eliciting any biological activity [35-37]. It probably opposed crucial effects of thyroid hormones during the differentiation of erythyroid cells. c-*erb*-A may thus be considered as an anti-oncogene since, when its biological activity is inhibited, some target cells become oriented towards a malignant phenotype.
Another line of research which led to similar conclusions regarding the relationship between intranuclear receptors and cancer was the search in human hepatomas for insertion sites of hepatitis virus DNA. In one patient, such a site was cloned and sequenced and

found to encode a polypeptide homologous to the DNA binding domain of steroid receptors [38]. The cloning of the corresponding cDNA led to the isolation of the proto-oncogene (normal cellular equivalent of the oncogene), which was subsequently found to bind retinoic acid. Two other types of retinoic acid receptors were later described [21-25]. It is likely that insertion of hepatitis virus DNA had activated the retinoic acid receptor gene and had led to the synthesis of an abnormal form of the receptor which elicited, at least partially, the malignant transformation of hepatic cells. The modified retinoic acid receptor thus played the role of an oncogene.

The family of nuclear receptors has been further extended by 2 types of observations. Firstly, cross-hybridising cDNA species were cloned and sequenced, showing the characteristic pattern of nuclear receptors, for which, however, the nature of the ligand was unknown [2,39]. These "orphan" receptors await discovery of their function. Secondly, several genetic loci have been located in drosophila which direct various stages of embryological development and for which cloning and sequencing of the corresponding genes has clearly shown that they belong also to the family of intranuclear receptors [26-28]. Since such genes are usually highly conserved during evolution, we may expect, in the near future, their cloning in mammalian cells. The study of their function may be of interest for the understanding of the differentiation and growth of various cell types and thus for the analysis of the mechanisms of their malignant transformation. It is, at present, unknown if the function of these proteins is controlled through the binding of a ligand.

Among this large family of nuclear transcriptional regulators, 2 subgroups may be defined by their very close structural analogy. One involves the receptors for glucocorticoids, progestins, mineralocorticoids and androgens (receptors for steroids having mainly a 3 keto Δ 4 structure in their A ring). All of these receptors share more than 80% homology in their DNA binding domain. This explains why, in many cases, they can modulate the function of the same hormone-responsive elements. For instance, all stimulate the transcription of Mouse Mammary Virus (MMTV) Long Terminal Repeat (LTR) promoter. The similarity of these receptors is also high in the steroid binding region (>50%), but is totally divergent in the N-terminal domain. Another subgroup involves the different thyroid hormone and retinoic acid receptors. The oestrogen receptor does not belong to any of these subgroups.

Chromosomal Localisation of Receptor Genes

All nuclear receptors seem to be derived from a common ancestor. It was thus of some surprise to find that they were scattered throughout the genome. For instance, the oestrogen receptor gene was present on chromosome 6q24-27 [40], the progesterone receptor gene on chromosome 11q22-23 [41], the glucocorticoid receptor gene on 5q-q32 [42], etc. Only some of the receptors for retinoic acid and thyroid hormones are clustered in the same regions of chromosomes 3 and 17 [43-45].

Receptor genes are very large, due to the presence of large introns. For instance, the oestrogen receptor gene is over 140 Kb long, and contains 8 exons [46]. An interesting feature is the fact that the two zinc fingers of the DNA binding domain are encoded by separate exons.

The structure of the promoters of the receptors has been described [47], and the mechanisms which direct their hormonal regulation and tissue-specific expression are currently analysed.

Posttranslational Modifications of the Receptors

Two types of receptor phosphorylation reactions have been described. For oestrogen receptors, Auricchio and coworkers [48] have observed a tyrosine phosphorylation, catalysed by a specific kinase, which seems to be a prerequisite for the receptor to bind the hormone. No similar results have been reported by other groups.

Serine phosphorylations have been observed for progesterone [49,40], glucocorti-

coid [51], vitamin D and oestrogen (G. Green, personal communication) receptors. It was observed that the progesterone receptor could undergo two successive phosphorylation reactions [49,52]: one basal in the absence of hormone and a second one, hormone dependent, which elicited a characteristic shift in receptor electrophoretic migration ("upshift"). The role of these phosphorylations and especially of the hormone-dependent phosphorylation is not clear. It does not seem to modify receptor interaction with hormone-responsive elements [53], but it may play a role in the subsequent modulation of target gene transcription. It may also be involved in receptor down-regulation mechanisms.

Receptor Interaction with Genes. Role of Hormones and Antagonists

Three types of contacts of regulatory protein with DNA have been described: the helix-turn-helix motif in which one of the alpha helices contacts the DNA, the leucine zipper in which the basic regions of 2 protein monomers are brought into proper alignment to contact DNA by interaction of a stretch of leucines (appearing with a periodicity of 1 in every 7 amino acids), and, finally, the zinc finger motif which is present in steroid receptors. In the zinc fingers, the DNA binding structure is formed either by 2 histidines and 2 cysteines or by 4 cysteines coordinated by a Zn^{2+} atom. Two such fingers, each composed of 4 cysteines, are present in the nuclear receptors [54,55].

The receptor interacts with specific DNA sequences called hormone-responsive elements (HREs) (review in [56]). For a given receptor, the sequences are never identical but do resemble each other enough to allow the definition of a consensus sequence for glucocorticoid/progesterone receptors (GGTACAnnnTGTTCT) or for oestrogen receptors (AGGTCAnnnTGACCT). These HREs have, in most cases, a palindromic structure, suggesting that the receptors should bind as dimers or tetramers, and dimerisation of receptors during binding to HREs has indeed been demonstrated [57-59]. The hormone-responsive elements lie in most cases (but not always) upstream from the site of initiation of transcription of the gene. Their distance from it is variable, ranging from less than 100 to several thousand base pairs. In most promoters, several regions binding receptors are found, and in some cases they have been shown to exert a cooperative activity, perhaps through receptor-receptor interactions [60]. The fact that HREs are *cis* elements exerting their effect regardless of their position or sense, allows them to be classified among enhancer elements. How binding to such enhancers modifies gene transcription is not understood, the most likely hypothesis being that contacts between receptors and transcription factors lead to increased initiation by RNA polymerase [61].

In some systems, the steroid does not have a stimulatory activity but, on the contrary, is an inhibitor of gene transcription [62,63]. In these cases, it has been shown that the steroid-receptor complex impedes the binding of a transcription factor (e.g., the COUP factor in the case of glucocorticoid receptor and pro-opiomelanocortin gene).

The exact mechanism by which steroids modulate these reactions is not clearly understood. *In vivo*, in the cell, the hormone is, of course, necessary for the receptor to be active. By *in vivo* footprinting it has also been shown that hormone is necessary for receptor binding to HREs [64], but, once purified, the receptor binds to HREs even in the absence of its ligand [53,65]. It was recently shown that the purified receptor regulates gene transcription in a cell-free system in the absence of hormone [66]. To explain these findings it has been proposed that the receptor *in vivo* interacts with an inhibitory factor which prevents its transformation into the active state [53]. The hormone modifies the confirmation of the receptor, provoking its dissociation from this factor and its subsequent activation. *In vitro*, after purification, the isolated receptor can undergo this change in conformation, even in the absence of hormone, since it has been dissociated from the putative inhibitory factor.

A candidate for the role of inhibitory factor is the heat shock protein 90 [67-69]. In low-ionic-strength cellular extracts, the receptor is bound to this protein; when activated it is free. This association may exist *in vivo* but it is still possible that it is an artifact of cellular ho-