An intriguing double act

Oestrogen receptors

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The birth of nuclear receptors took place at the end of the 1950s when Elwood Jensen discovered specific binding of tritium-labelled oestradiol-17β in the uterus, a known target of oestrogens. Over the following years, Jensen and collaborators, e.g. Jacobsen and Gorski, identified the oestradiol-concentrating mechanism as a high-affinity low-capacity soluble receptor which was termed the ER (oestrogen receptor)[1].

Soon, receptors for other steroid hormones were identified. O'Malley performed ground-breaking studies on the progesterone receptor and, on the basis of the results of Jensen, O'Malley and others, the hypothesis was formulated that, following entrance of the steroid hormone into the cell, a cytoplasmatic steroid–receptor complex was formed. This complex also contained a dimer of the heat-shock protein, hsp90, which served to keep the receptor in a conformation allowing binding of ligand. After an energy-dependent process, the heat-shock protein dissociated from the receptor whereupon the receptor entered the cell nucleus and bound to DNA[2]. Later, this chain of events was reinterpreted and it has become clear that most steroid hormone receptors actually reside in the cell nucleus and that the steroid hormone travels directly through the cytoplasm to the cell nucleus (Figure 1). Accordingly, steroid receptors are now named nuclear receptors.

Interestingly, the transport mechanism(s) participating in the transcellular journey of the steroid receptors is (are) still poorly understood. It has been suggested that the receptors might glide along some type of fibres constituting a cytoplasmatic network stretching from the cell membrane to the cell nucleus, but such a mechanism has not been proved convincingly. Another unresolved aspect of nuclear receptors is the issue of whether these receptors can reside in the plasma membrane of the cell. The issue remains controversial and will remain so until such entities have been purified and characterized.

Once the nuclear receptor has bound its ligand, it interacts with specific sites on DNA, usually in the vicinity of regulated genes, and this interaction initiates a series of events that finally results in an altered rate of transcription of the regulated genes. The first insights into the mechanism of nuclear receptor interaction with DNA came from a collaboration between our own laboratory and that of Keith Yamamoto at UCSF (University of California San Francisco). In this collaboration, we provided purified glucocorticoid receptor from rat liver, while Yamamoto and associates contributed glucocorticoid regulated murine mammary tumour virus. Together we could show that the glucocorticoid receptor bound specifically to certain regions of the tumour viral DNA. These regions turned out to constitute glucocorticoid-response elements, which conferred glucocorticoid sensitivity on to heterologous genes when they were inserted upstream of their promoters[3].

The mechanisms of nuclear receptor actions are similar to those of other DNA-binding transcription factors and today a very detailed understanding has been obtained of the many steps that constitute the transcriptional response. What is unique about most nuclear receptors is that they do not bind to DNA unless they are activated by ligands, which are low-molecular-mass substances (steroid hormones, fatty acids, oxysterols, vitamins etc.) which have specific high affinities for the ligand-binding pocket in the C-terminal domain of the different nuclear receptors. The finding that such small molecules may turn target genes on or off via activation of nuclear receptors was a turning point in our understanding of the mechanism of gene regulation by hormones, dietary components and metabolites. The binding of nuclear receptors to DNA occurs via a specific domain located in the centre of the receptor and called the DNA-binding domain. At the N-terminus of the receptor is a domain that harbours functions that participate in its transcriptional activities[4].

In addition to the transcriptional effects of the steroid–steroid hormone receptor complex which take at least several minutes to result in biologically evident effects (changed expression of RNA and proteins), there are more rapid actions of steroid hormones, the so called non-genomic actions of steroids (Figure 1). The most well-studied of these effects are the electrophysiological changes in the CNS (central nervous system) that occur within seconds[5]. Much less is known about the mechanism of these extranuclear effects of steroid hormones and nuclear receptors than about their effects on gene transcription. In recent literature, convincing reports are beginning to appear that make it likely that, indeed, certain nuclear receptors may associate with cellular membranes and exert specific biological effects by changing the properties of these membranes.

Key words: *DNA, insulin resistance, ligand, nuclear receptor, oestrogen, steroid*

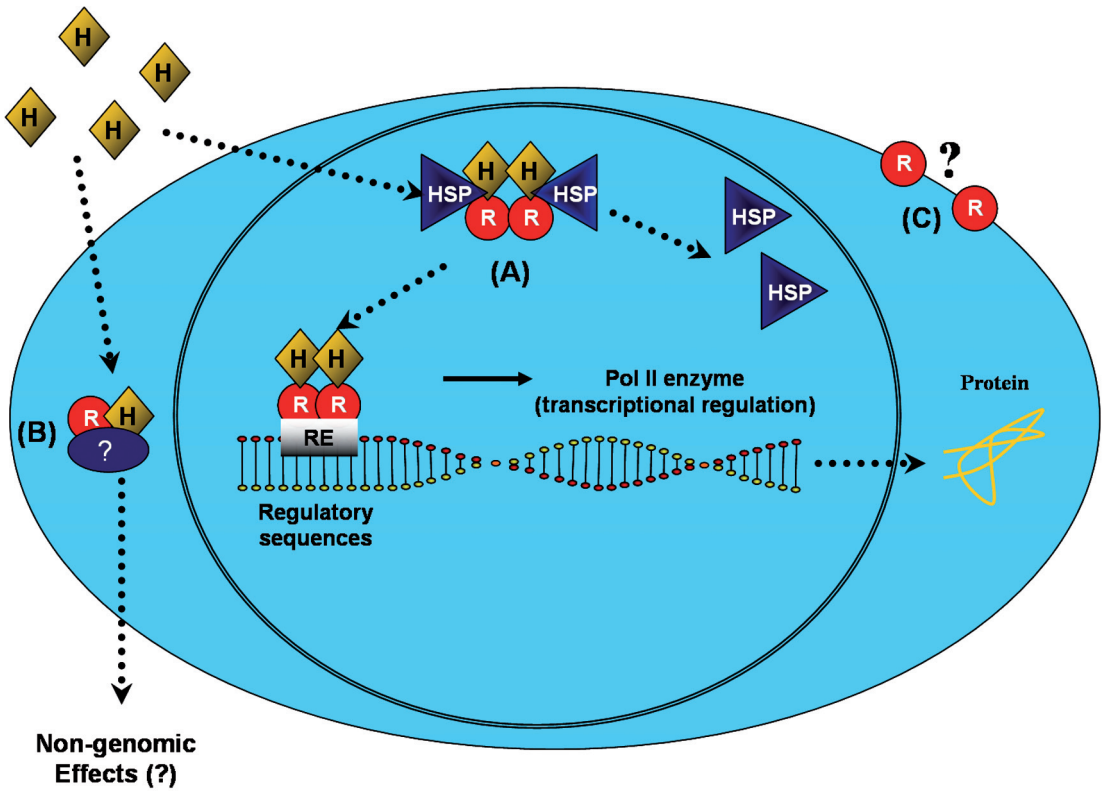

Figure 1. Mechanism of steroid action. Following entrance of the steroid hormone (H) into the cell, a complex constituting H, steroid-receptor (R) and heat-shock proteins (HSP) is formed in the nucleus. After an energy-dependent process, the HSP dissociates from the complex and the remaining dimer of H–R binds to specific sequences of the DNA named response elements (RE). This interaction alters the activity of the polymerase II (Pol II) enzyme, modulating the rate of transcription of the target genes and, consequently, protein synthesis (A). In addition to the nuclear transcriptional effects, non-genomic actions of steroids seem to take place in the cytoplasm, where the H–R complex binds to proteins and cause response within minutes of interaction. This mechanism, however, is less well understood than the transcriptional actions of steroids (B). The presence of R on the cell membrane has been debated over the years; it is possible that H–R may alter the property of cell membranes and initiate a cascade of biochemical events (C)

Oestrogen receptors

It was long thought that there was only one ER. It was therefore almost a shocking discovery to the endocrine community when our laboratory reported on a second ER, ERβ, which we isolated from rodent ventral prostate[6]. We renamed the 'Jensen receptor' ERα (Figure 2). Our finding constituted a real paradigm shift in the understanding of oestrogen action. In the following years, we showed that the two ERs often had opposite actions, in a 'yin–yang' fashion (Figure 3). For instance, whereas ERα is involved in proliferative actions of oestrogens in target tissues such as mammary gland and endometrium, ERβ often displays antiproliferative properties in these and other tissues. This has led to hopes that ERβ might be a 'druggable' target in therapy against certain forms of cancer.

The two ERs are widely distributed in the body, reflecting the diverse actions of oestrogen signalling in physiology (Figure 4). This is also reflected in the fact that inactivation of the ER genes leads to phenotypes in most tissues in the body. That is, ERs are active not only in reproductive tissues, but also in several other organs, e.g. the central nervous system, immune system, gastrointestinal tract, lung, pancreas and urinary bladder. Below a few specific examples are given with regard to interactions between ERα and ERβ in different tissues.

In the brain, both ERα and ERβ are expressed with overlapping, but specific, localization in different areas[7]. Studies on mice with deletion of one or other of the ERs have helped delineate the different functions of the two ERs in the CNS. It appears that ERα is the main ER involved in regulation of sexual behaviour, whereas ERβ is more important in regulating social behaviour. Interestingly, ERβ seems to balance the aggressive component in ERα-induced sex behaviour (yin–yang principle); in

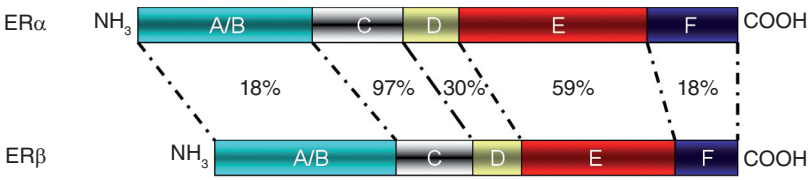

Figure 2. Linear structure of human ERα and ERβ. ERs are composed of five domains: A/B domain at N-terminal region, highly variable in length and sequence and involved in protein–protein interactions; DNA-binding domain (C), highly conserved and with a motif responsible for DNA binding; hinge domain (D), with a nuclear localization signal and important for the three-dimensional structure of the receptors; ligand-binding domain (E), forming the ligand-binding pocket and associated with dimerization and interaction with cofactors; and C-terminal domain (F), highly variable and involved in transactivation of the receptors. The percentages indicate the homology between ERα and ERβ. The low percentage observed in the A/B domain (18%) suggests that ERα and ERβ interact differently with proteins

line with this, ERβ agonists have anxiolytic effects. Furthermore, ERβ seems to be the main ER involved in the antidepressive effects of oestrogen; these effects are thought to be mediated through the raphe nucleus where ERβ dominates over ERα and where 5-hydroxytryptamine (serotonin) levels are increased by oestrogen.

In the normal mammary gland, ERβ is the predominant ER, but in breast cancer, ERα becomes the dominating receptor[8]. Whereas ERα is a commonly used biomarker in breast cancer, signalling sensitivity to hormonal treatment and indicating a good prognosis, the significance of ERβ in breast cancer is currently less well understood. An increasing number of studies are suggesting that even expression of ERβ signals a good prognosis, but there are also investigations where such an association has not been found. A possible explanation for this confusion might be that commercially available antibodies against ERβ, necessary for measurement of ERβ protein, often have been of highly variable quality and have therefore resulted in unreliable assays of ERβ. Accordingly, further studies are needed before the value of ERβ assays in breast cancer may be assessed.

The prostate gland is the tissue in males which is richest in ERβ[8]. The receptor is abundantly expressed in the epithelium, while the stroma contains small amounts of ERα. Deletion of ERβ results in hyperplasia of the prostate and, later on, in cancer *in situ* (PIN, prostatic intraepithelial neoplasia). Accordingly, ERβ appears to be antiproliferative not only in the mammary gland, but also in the prostate. Like in breast cancer, ERβ gradually diminishes in concentration as the prostate cancer progresses. When ERβ is introduced into breast or prostate cancer cell cultures, it blocks cell growth, i.e. its actions are opposite to those of ERα which stimulates cell growth, another example of the yin–yang relationship between the two receptors.

Most studies show that expression of ERβ decreases during carcinogenesis in both the prostate and the mammary gland. This decrease appears to be the consequence of methylation of the promoter of the ERβ gene. Consequently, attempts to treat breast and prostate cancer with ERβ agonists need to be combined with administration of demethylating agents. Recent studies show that in prostate cancer cell lines, treatment with valproic acid (a demethylating agent) results in restoration of ERβ expression and increased sensitivity to the antiproliferative effects of phytoestrogens.

It has been suggested that certain diets rich in phytoestrogens, such as soya and tofu, might protect against prostate cancer. Interestingly, these non-steroidal compounds bind better to ERβ than to ERα. It may well be so that phytoestrogen intake may keep ERβ activated and therefore exert a protective influence against growth of the prostate gland.

The gastrointestinal tract is lined with epithelium expressing ERβ, especially in the stomach and colon[8]. Interestingly, hormone-replacement therapy in post-menopausal women seems to reduce the risk of gastric cancer and colon cancer by 50 and 30% respectively. Thus it appears as though ERβ exerts an antiproliferative effect in these contexts as well. When microarray analysis is carried out on colon cancer cells in culture, before and after expression of recombinant ERβ, this receptor affects the activities of several hundred genes in such a way that genes involved in tumour promotion (e.g. oncogenes) are suppressed, whereas genes active in slowing down cellular growth (e.g. tumour-suppressor genes) are up-regulated.

Also other forms of cancer are seen in mice with ERβ deleted, such as pituitary and ovarian tumours, which indicates important roles of ERβ in the hypothalamo–pituitary–ovarian axis. Current studies in our laboratory indicate that ERβ activates the tumour-inhibitory wing of TGFβ (transforming growth factor β), by up-regulating inhibin and its receptor, TGFβR3, also called β-glycan. Deletion of ERβ would thus lead to uninhibited activin which, together with FSH (follicle-stimulating hormone) and in the absence of antiproliferative ERβ, gives rise to a multitude of ovarian tumour types such as granulosa cell and ovarian surface epithelial tumours, often with a high mitotic index.

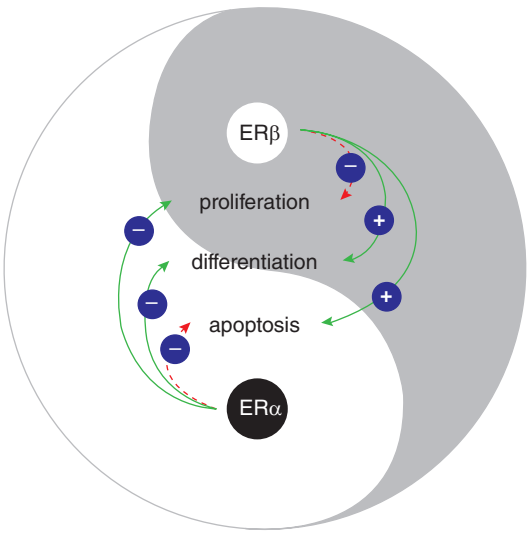

Figure 3. The 'yin–yang' relationship between ERα and ERβ. One important action of ERβ is to regulate ERα-mediated activity. Whereas ERα decreases apoptosis and cell differentiation and increases cell proliferation, ERβ has opposite actions. It increases apoptosis and cell differentiation and decreases cell proliferation. This balance between ERα and ERβ in tissues where both receptors are expressed has important consequences in physiology and disease

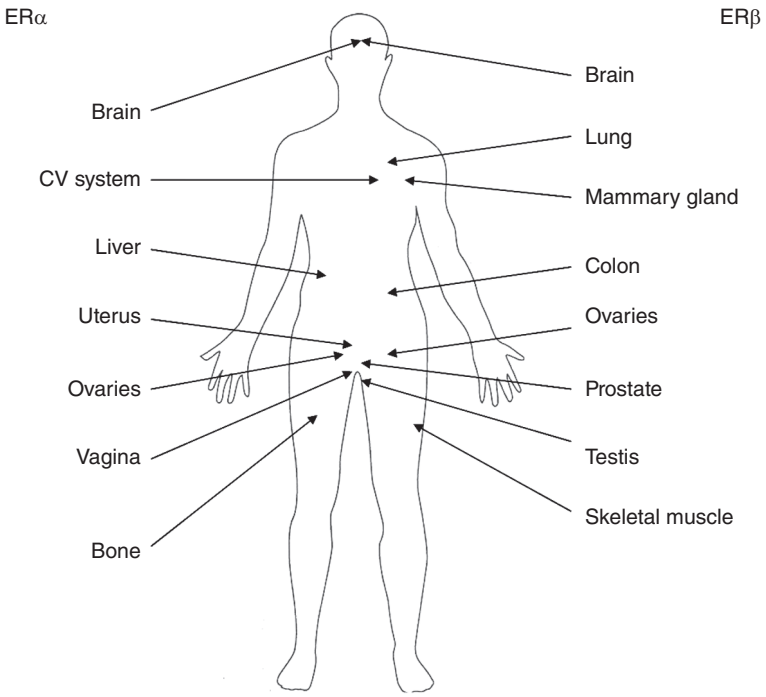

Figure 4. Tissue distribution of ERα and ERβ. ERα is mainly expressed in tissues of classical oestradiol actions, such as uterus, vagina, ovaries, but is also present in brain, liver, bone and cardiovascular (CV) system. ERβ is more widely distributed and is present in brain, lungs, mammary glands, colon, prostate, testis, ovaries and skeletal muscle. Some tissues, such as mammary glands, uterus, liver, brain and skeletal muscle, express both ERs, with the predominance of one or the other isoform. The final result of their actions will depend on which isoform is predominant

The ovary is the organ in females which is richest in ERβ[8]. The receptor is expressed in the granulosa cells and is important for maturation of oocytes and ovulation. Deletion of ERβ results in severe infertility and in an ovarian phenotype somewhat reminiscent of that in polycystic ovarian dysfunction. Studies in our laboratory indicate that aberrations in vascularization of the growing follicles results from a lack of remodelling of the surrounding thecal layer. Possibly, ERβ agonists may be of use in the treatment of some forms of infertility in females.

As a reflection of the paramount importance of oestrogen signalling in many biological systems, intermediary metabolism is also regulated by the balance between ERα/ERβ signalling. Mice with deleted ERα develop insulin resistance, obesity and glucose intolerance[9]. Ovariectomy of these mice normalizes these symptoms, indicating that overactive ERβ may be involved in causing the metabolic syndrome. This notion is supported by the finding that ERβ down-regulates the glucose transporter GLUT4 in skeletal muscle[10].

In conclusion, oestrogen signalling regulates important physiological events throughout the organism, perhaps a reflection of the fact that ERs may have been the first steroid receptors to have appeared during evolution. In particular, the discovery of ERβ and the ERα/ERβ paradigm have clarified the mechanism behind several oestrogen-associated pathologies which were previously difficult to explain, i.e. the fact that oestrogen may exert completely opposite effects depending upon the cellular context and the ratio between ERα and ERβ. Work is now ongoing in several drug companies to attempt to use ERβ agonists in the treatment of various disorders, and current clinical trials will soon tell us whether these attempts will turn out to be fruitful. ■

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