# Steroid hormone action mechanisms

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Cellular signaling constitutes an important component of information flow in biological systems. It has been evolutionarily conserved from micro-organisms to humans. All such organisms use one or the other form of signal(s) to generate the desired response. These signals include a wide variety of molecules starting from amino acids and their derivatives to proteins on one hand and the steroids and other lipid derivatives on the other. The hydrophilic signals (amino acid derivatives and proteins), being water soluble, cannot cross the plasma membrane and hence act by binding to specific membrane-bound receptors. These receptors are mostly coupled to transducer G-proteins which influence the amplifier enzymes to produce a variety of second messengers (cAMP, cGMP, $IP_3$, DAG, $Ca^{2+}$, etc.). The second messengers thus generated, modify the effector proteins and enzymes to elicit the cellular response[1]. On the other hand, the lipophilic signals (steroids and their derivatives), being lipid soluble, can cross the plasma membrane and bind to specific intracellular receptors, located either in the cytosol (for glucocorticoids) or in the nucleus[1] (for sex steroids, thyroid hormones, vit. D3 and retinoic acid). After the initial discovery of receptors for steroid hormones during 1960s, many controversies arose which were subsequently solved to a large extent[2]. Originally these steroid receptors were discovered in the cytosol and thereafter they were also found to be present in the nucleus of target cells. This was during mid 1980s when the arrival of monoclonal antibodies to specific steroid receptors led to the observation that the glucocorticoid receptors are primarily localized in the cytosol, whereas sex steroids in the nucleus of target cells[3]. Binding of the steroid and related hormones led to a conformational change in the receptor molecules, thereby converting a non-DNA binding form of the receptor to a DNA-binding form. This process is termed as activation or transformation of the receptor. Association of the heat shock proteins along with a number of other well-characterized modulators with the untransformed receptors has opened a new dimension to the steroid hormone action mechanisms. These modulators maintain the inactive state of the receptors and the binding of steroids leads to dissociation of these chaperones that converts the receptors into a DNA-binding form[4,5]. Activated steroid–receptor complexes then interact with specific DNA sequences, usually located a couple of hundred base-pairs upstream the regulated gene, and modulate its expression (genomic action) by influencing the synthesis of mRNAs and respective proteins, thus producing the cellular response[6] (Figure 1). These intracellular steroid receptors act like ligand-activated transcription factors. They consist of a variable *N*-terminus that contributes to the transactivation function; a highly conserved DNA-binding domain responsible for specific DNA-binding and dimerization and a C-terminal domain involved in ligand-binding, nuclear localization, and ligand-dependent transactivation function[6].

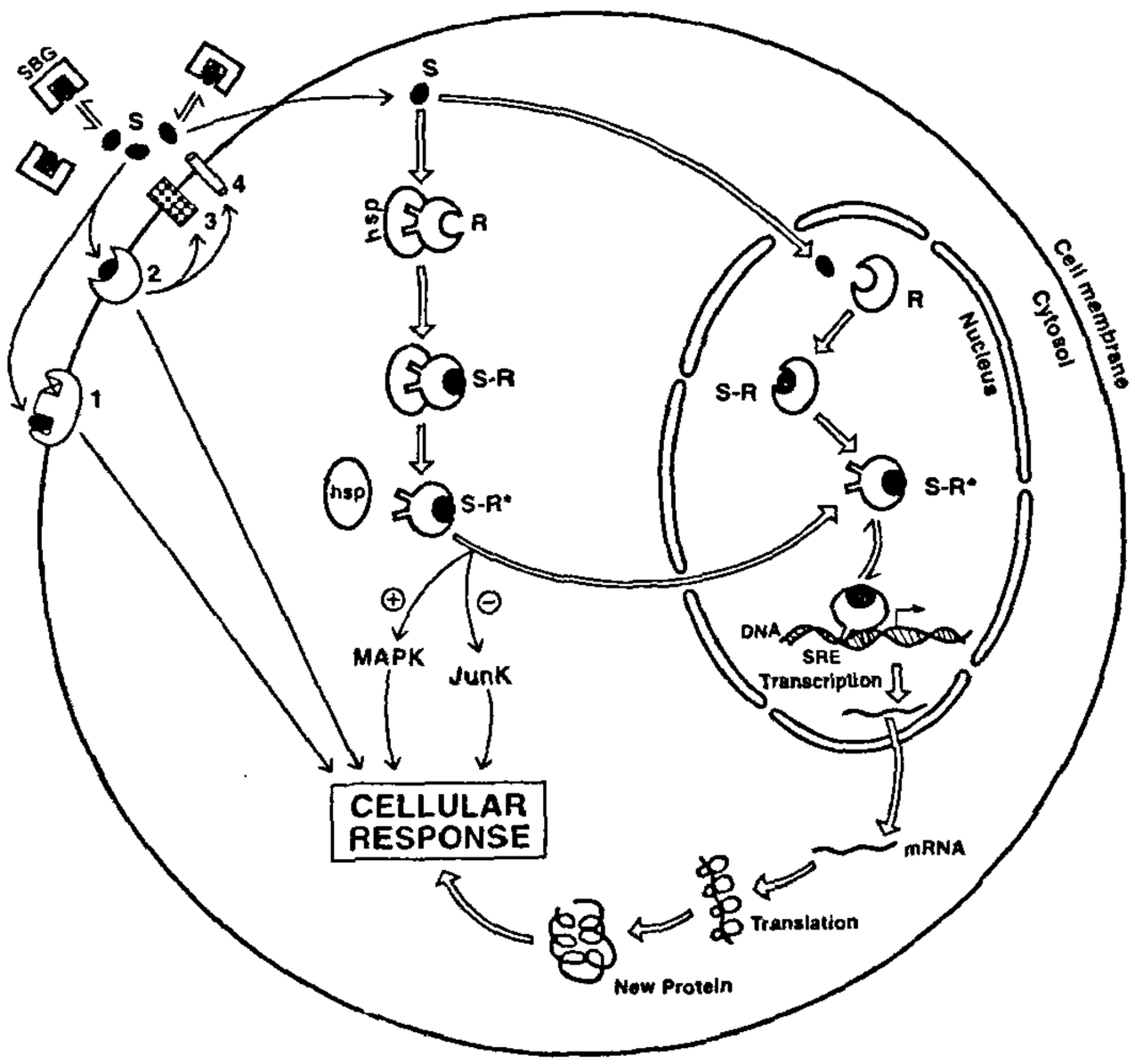


**Figure 1.** Multitude of steroid hormone action mechanisms. Steroids (S) primarily act through intracellular receptors (R), located either in the cytosol or in the nucleus of a *target cell. Steroid–receptor (S–R) complexes undergo activation** (transformation) and interact to specific DNA sequences, termed as steroid–responsive elements (SREs). This interaction modulates the cognate gene expression by influencing the synthesis of mRNAs and related proteins, thus producing an appropriate cellular response. In addition, steroids may also influence cellular responses through cell-surface receptors. These membrane-bound receptors may either be specific to steroid (2) or steroids may bind to other protein/peptide hormone receptors (1). Interaction to the latter may modulate the signaling of the respective protein/peptide hormone; while the binding of steroid to specific membrane receptor (2) may directly or indirectly influence the cellular responses either through membrane associated protein tyrosine kinase (3) or through associated ion channels (4). [⇒, genomic actions; → non-genomic actions; SBG, steroid binding globulin; hsp, heat shock protein; MAPK, mitogen-activated protein kinase; JunK, Jun kinase; +, stimulation; –, inhibition; ☿, protein/peptide binding site; ●, steroid binding site].

This view of action mechanism for the two distinct classes of hormones prevailed up to the late 1980s and was mostly considered independent of each other. Later, the idea of cross-talk between the intracellular steroid action cascade and the cell-surface protein/peptide hormone action cascade arose and visualized the inter-relations among the protein/peptide and steroid hormone actions[7]. Steroid hormone action can be modulated by the protein/peptide hormone modifiers. We have earlier reported that the protein kinase C activators and inhibitors modulate the glucocorticoid-dependent regulation of tyrosine aminotransferase and tryptophan oxygenase in cultured rat hepatocytes[8,9]. Several others have also observed that the protein kinases are central to these cross-talks, as most of the steroid receptors are phosphoproteins and their phosphorylation might control the activation and affinity of these receptors to DNA response elements. Selected steroid receptors can be activated in a ligand-independent manner by a membrane receptor agonist. Dopamine has been reported to mimic the action of progesterone in activating the progesterone receptors while 8-bromo-cAMP has been demonstrated to mediate progesterone receptor-dependent transcription in the absence of progesterone[10,11].

More recently, receptors for steroid hormones were also reported to be located on the membrane surfaces of certain cell types such as spermatozoa, oocytes, endometrial cells and granulosa cells[12]. The non-genomic effects of 17$\beta$-estradiol, progesterone, testosterone and androstenedione on these reproductive cell types are well presented[12]. They influence the production of intracellular second messengers such as $Ca^{2+}$ and $IP_3$ in a very rapid manner, too fast to be mediated by the sequence of genomic activation. These are some of the steroid actions which may not necessarily be explained by the genomic actions as stated above, particularly those of the rapid ones where no mRNA and protein syntheses are observed. Some of these non-genomic actions of the steroid hormones are not blocked by the inhibitors of transcription as well as translation. These effects are also not abolished by the antagonists to the genomic receptors. Such non-genomic actions of steroid hormones are mediated by the binding of steroids either to its own specific cell-surface receptor or through interaction with other protein/peptide hormone receptor[13]. If the steroid knocks at the latter, it modulates the effectiveness of the concerned protein/peptide hormone. However, if it knocks the former, it influences directly or indirectly the membrane-bound protein tyrosine kinases or the associated ion channels. Both these culminate to a cellular response[12,13]. There are specific examples to support the view that steroids need not always have to elicit changes in the gene expression. Progesterone, an essential progestational hormone of corpus luteum, maintains pregnancy in mammals and is opposed by oxytocin, a nonapeptide that induces uterine contraction and facilitates labour and parturition. One of the functions of progesterone is to maintain the uterus in a quiescent state by decreasing the sensitivity of uterus to oxytocin. Although it is mostly held that progesterone acts at a genomic level by interacting with the nuclear receptor and modulating the cognate gene expression, Grazzini *et al.*[14] have recently shown that progesterone inhibits oxytocin signaling by binding to the membrane-bound oxytocin receptor and changing the conformation such that oxytocin does not interact effectively to its own receptor. The oxytocin receptor belongs to a large class of membrane-bound receptors that relay their signals through G-proteins to amplifier enzymes such as phospholipase C. Progesterone binding to cell surface membranes of rat oxytocin receptor-expressing CHO cells inhibits the production of $IP_3$ and intracellular $Ca^{2+}$ concentration[14]. This non-genomic action of progesterone is obtained in less than a minute and is readily reversible. Progesterone might bind to an allosteric site of oxytocin receptor and produce conformational change that prevents oxytocin from binding to its cognate site. Their findings provide the first evidence for a direct interaction between a steroid hormone and a G-protein-coupled receptor and added to a new stage of cross-talk between the peptide and steroid hormone signaling cascade[14]. In fact, this is not the lone example of non-genomic action of steroids through membrane-bound receptors. Progesterone also seems to mediate a variety of biological processes, both stimulatory and inhibitory, through non-genomic actions by interacting with uncharacterized associations to receptors for the neurotransmitters GABA, *N*-methyl-D-aspartate (NMDA) and acetylcholine[15,16]. Even the reports on progesterone modulating membrane associated protein tyrosine kinases as well as $Ca^{2+}$ channels in human spermatozoa have appeared recently[12] (Figure 1). These findings clearly open another gate of signaling by the steroid hormones, may be in specific cases.

In addition, some of these steroids even inside a cell, affect signaling pathways in a transcription-independent manner through their cognate intracellular receptors. Estrogen receptor activates the mitogen-activated protein kinase (MAPK) signaling that is turned on by mitogen-activating protein factors[17]. Glucocorticoid receptor interferes with the activation of a related signal cascade by ultraviolet and inflammatory signals[18]. Taken together the genomic, non-genomic, and cross-talks in the steroid and protein/peptide hormone actions, it will be of immense benefit to modulate specific events in the steroid signaling and develop drugs that can effectively block a particular mode of steroid action. It seems almost certain that the active genomic steroid action mechanism coexists with the non-genomic actions by the same ligand that can simultaneously work to achieve the desired cellular response. The details of the relative contributions of both genomic and non-genomic steroid action mechanisms together with the cross-talk among them are yet to be appropriately exploited.


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## COMMENTARY

# Can great discoveries be orchestrated?

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Many science administrators of India and also Directors of many Institutes have often wondered whether it will be possible to orchestrate great discoveries from this country. It may be relevant to give a few paragraphs from an essay written by Max Perutz, one of the most outstanding scientists of this century.

'Every now and then I receive visits from earnest men and women armed with questionnaires and tape recorders who want to find out what made the Laboratory of Molecular Biology in Cambridge (where I work) so remarkably creative. They come from the social sciences and seek their Holy Grail in interdisciplinary organization. I feel tempted to draw their attention to 15th century Florence with a population of less than 50,000, from which emerged Leonardo, Michelangelo, Raphael, Ghiberti, Brunelleschi, Alberti, and other great artists. Had my questioners investigated whether the rulers of Florence had created an interdisciplinary organization of painters, sculptors, architects, and poets to bring to life this flowering of great art? Or had they found out how the 19th century municipality of Paris had planned Impressionism, so as to produce Renoir, Cézanne, Monet, Manet, Toulouse-Lautrec, and Seurat? My questions are not as absurd as they seem, because creativity in science, as in the arts, cannot be organized. It arises spontaneously from individual talent. Well-run laboratories can foster it, but hierarchical organization, inflexible, bureaucratic rules, and mountains of futile paperwork can kill it. Discoveries cannot be planned; they pop up, like Puck, in unexpected corners.

'In the past, most scientists were poorly paid; only few became famous and even fewer rich. One of the characters in Fred Hoyle's novel *The Black Cloud* remarks that scientists are always wrong, yet they always go on. What makes them continue? Often it is addiction to puzzle-solving and ambition to be recognized by their peers.

'Science has changed the world, but the scientists who changed it rarely foresaw the revolutions to which their research would lead. Oswald Avery never set out to discover what genes are made of; Hahn and Meitner never intended to split the uranium nucleus; Watson and Crick were taken by surprise when their atomic model of DNA told them how the genetic information replicates itself; and when Jean Weigle and Werner Arber wondered why a bacterial virus infected one strain of coli bacteria and not another, they could not foresee that some 40 years on, their enquiry would lead to the cloning of a sheep named Dolly. Like children out on a treasure hunt, scientists don't know what they will find.

'According to Paul Ehrlich, the father of immunology, scientists need the four Gs: Geschick, Geduld, Geld, und Glück (skill, patience, money, and luck). Patience may or may not reap its own reward. The astronomer Fritz Zwicky built a new kind of 18-inch telescope at Mount Palomar in California in order to obtain images over a wide field of the sky. He wanted to scan these images for exploding stars, supernovae which flare up suddenly and can be brighter than a million suns. Between September 1936 and May 1937, Zwicky took 300 photographs in which he scanned between 5000 and 10,000 nebular images for new stars. This led him to the discovery of one supernova, revealing the final dramatic moment in the death of a star. Zwicky could say, like Ferdinand in *The Tempest* when he had to hew wood:

> For some sports are painful and the labour
> Delight in them sets off; some kinds of baseness
> Are nobly undergone, and most poor matters
> Point to rich ends. This my mean task
> Would be as heavy to me as odious; but
> The mistress which I serve quickens what's dead
> And makes my labours pleasures.

'The heavens were Zwicky's mistress, and mine was hemoglobin, the protein of the red blood cells. As part of my attempt to solve its structure, I took several hundred X-ray diffraction pictures of hemoglobin crystals, each taking two hours exposure. I took some of the pictures during World War II, when I had to spend nights in the laboratory in order to extinguish incendiary bombs in the event of a German air raid. I used these nights to get up every two hours, turn my crystal by a few degrees, develop the exposed films and insert a new packs of films into the cassette. When all the photographs had been taken, the real labour only began. Each of them contained several hundred little black spots whose degree of blackness I had to measure by eye, one by one. After six years of this labour, when the data were finally complete, a London firm processed