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Minireview

Receptors for mammalian steroid hormones in microbes and plants

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Steroids are of universal occurrence, present variously as cell wall constituents and bioregulators. A number of bacteria, fungi, and photosynthetic vascular plants synthesize steroids that are hormonally active in the animal world. The cellular effect of such steroids in microbes and plants appears by and large to be comparable to that in mammals. Available evidence suggests that steroid action in botanical phyla is mediated via receptors organized in a manner similar to that seen in animals. Therefore, the ancestry of ligand induced transactivation via zinc finger proteins appears to date farther back than the early Cambrian burst of metazoan evolution 500 million years ago.

Microbe; Plant; Steroid; Hormone; Receptor

1. STEROID DISTRIBUTION AND SYNTHESIS

Receptor mediated control of transcription by steroid hormones (Fig. 1) is a subject of much contemporary interest in mammalian biology. In microbes and plants, the evidence for distribution, synthesis, and physiological action, of steroids has remained rather circumscribed, and sometimes even contradictory. This minireview traces the role played by steroids as messengers at various levels of evolution, as a prelude to understanding receptor origin for further experimental inquiry.

Life on earth is believed to have originated some 4 billion years ago from about 30 biologically useful precursors, formed from simple inorganic matter under the influence of the cosmic energy reaching our planet. Steroids are of universal occurrence, present in the cell variously as membrane constituents, chemical messengers, vitamins, cytotoxins, and hormones [1,2].

Whereas prokaryotic transformation of steroids is rich in diversity [3], the synthesis of sterols follows two major routes in higher organisms [4]. Squalene 2,3-oxide, formed from acetate via common pathways, is cyclized either to lanosterol in non-photosynthetic eukaryotes (fungi, animals), or to cycloartenol in photosynthetic phyla [4]. Insects transform steroids and sterols ingested during feeding [2]. Plant sterols, saponins, cardiac glycosides, and alkaloids are of much biomedical value, but have no demonstrated counterparts or hormonal role in mammals [5]. Several bacterial and plant species synthesize cholesterol which is then transformed to steroids hormonally active in animals and plants [6–8].

Cholesterol is the common starting material in animals for P450 mediated synthesis of all classes of steroid hormones [9]. Plant species have been variously shown to contain glucocorticoids (cortisol), mineralocorticoids (deoxycorticosterone), progestins (progesterone), androgens (testosterone), estrogens (estradiol and estrone) and even the insect moulting hormone ecdysone [2,5–7,10,19–21], as shown in Table I.

2. CELLULAR FUNCTION OF STEROIDS

Earlier studies had shown that bacteria, mycoplasma (PPLO), and fungi respond to sterols and steroids in bewildering ways, ranging from vitamin-like to antimicrobial [7]. Mammalian gonadal hormones generally inhibit the growth of bacteria, in particular the Gram-positive organisms [7,11]. Deoxycorticosterone is most effective in growth inhibition assays [7,12], possibly due to its effect on Na–K exchange across the cell membrane of bacteria [7] and yeast [12,13], much as in mammals [14], and this can be specifically reversed by estradiol in a number of tests [7]. The growth of *Chlamydia trachomatis* is stimulated by cortisol [15], but this steroid appears ineffective in bacterial cells [7].

In *Achlya*, the fungal sex hormone, antheridiol, increases RNA and protein synthesis which is inhibited by actinomycin-D, as in mammalian systems [16]. Antheridiol shares structural homology with insect (ecdysone) and plant (abscissic acid) hormones, permitting a unified theory of function [16]. Dose- and time-dependent inhibition of *Trichophyton* growth in vitro by progestins, and deoxycorticosterone has also been

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Table I

Hormonal steroids in microbes and plants

Cholesterol	Digitalis lanata	Podocarpus
	Digitalis purpurea	Red algae
	Haplopappus heterophyllus	Slime molds
	Labyrinthula minuta	Tetrahymena colissi
	Malus sylvestris	Trypanosoma cruzi
	Mycoplasma laidlawii	
Corticosterone	Mallotus paniculatus	
Deoxycorticosterone	Digitalis lanata	
	Fusarium solani	
	Oryza sativa	
Estradiol	Butea superba	Prunus armeniaca
	Chenopodium rubrum	Trifolium subterraneum
	Elaies guineensis	Willow catkins
	Phaseolus vulgaris	
Estrone	Apple and bean seed	Phoenix dactylifera
	Clossostemon bruguieri	Prunus armeniaca
	Hyocyanus species	Punica granatum
	Mallus sylvestris	
Pregnenolone	Digitalis lanata	Nicotiana tabacum
	Digitalis purpurea	Trachycalymna fimbriatum
	Haplopappus heterophyllus	Xysmalobium undulatum
Progesterone	Aspergillus niger	Hollarrhena floribunda
	Curvularia lunata	Nicotiana tabacum
	Cheiranthus cheiri	Ophiobolus herpotrichus
	Digitalis lanata	Paecilomyces species
	Digitalis purpurea	Penicillium notatum
	Dioscorea deltoidea	Rhizopus nigricans
	Fusarium oxysporum	Strophantus kombé
	Fusarium solani	Trichoderma vividae
Testosterone	Pinus sylvestris	Pinus vulgaris

This table lists naturally occurring animal type of steroids in microbes and plants [2,5–7,10,19–21]. Many plants also contain hormonally active material having no structural analogy with animal steroids [19]. Enzymatic transformation can lead to the synthesis of other, structurally diverse, steroids [3]. Cortisol and aldosterone are apparently restricted to the vertebrate kingdom but their synthesis in other phyla can not be excluded.

shown [17], in contrast to growth stimulation of *Coccidioides* by several mammalian sex steroids [18].

Three-quarters of a century ago it was observed that many plant extracts possess estrogenic activity, leading to estrus in grazing animals. Some of these materials structurally resemble mammalian estrogens [19]. Topical application of androgens and estrogens variously affects flower formation in a number of plant species [20,21]. Mammalian sex steroids and cortisol also alter seed growth and vegetative development [20,21]. Inhibitors of steroid synthesis retard flowering whereas steroid derivatives in alpha configuration are biologically inert [21].

Recent data from this laboratory have gone farther by showing that the spirolactone RU 26752, known specifically to antagonize receptor-mediated mineralocorticoid action in mammals, inhibits the growth of *Chlamydomonas*, and this is fully reversed by the natural hormone aldosterone [22]. Cells surviving the inhibitory action of RU 26752 are afterwards refractory to this spirolactone, possibly due to an enzymatic adaptation, or a mutant receptor, or both [23]. This effect in a photosynthetic alga recalls growth inhibition by deoxycorticosterone in nonphotosynthetic fungi and bacteria [7,12], where Na/K balance is perturbed [24].

The nearly universal presence of sterols in cell and/or plasma envelop suggested that steroid-mediated effects could proceed via membrane bound carriers [24]. Membrane binding of androgens [25], mineralocorticoids [26], and progestins [27] in mammals have found a counterpart in bacteria [28], but further molecular elucidation of the mechanism is needed. In fact, the immediate, non-nuclear, action of preformed membrane-bound carriers could well complement transcription regulation, after a lag phase, by intracellular receptors [26,27].

3. RECEPTOR-MEDIATED TRANSACTIVATION OF GENES

It is generally accepted that, in the animal world, steroid hormones bind to specific receptors inside the cell, albeit the actual localization of these proteins in the resting state has become a matter of some controversy ([29] and references therein). In the presence of the cognate steroid ligand, the receptor assumes the conformation required for activation of transcription via zinc fingers identified to date in about 32 proteins (Fig. 1). Steroids can activate transcription of yeast [30,31] and plant [32] genes in chimaeric constructs in vitro, but evidence for the possible presence of such receptors in plants is rather scanty.

The binding pattern of estrogens, progestins, and glucocorticoids, in the cytosol of pathogenic or saprophytic fungi, does not resemble that for mammalian receptors, and is more in keeping with the binding of steroids to globulins in the blood [33]. Proteins of various sizes, with specificity for mammalian steroids, from *Candida* and *Saccharomyces* [33], and for antheridiol from *Achlya* [34], have been partially characterized by chromatography. Similarly, *Trichomonas* was reported to contain high affinity, low capacity, receptors for androgens as well as estrogens [35]. The biological significance of these steroid-binding proteins from fungi, and their relationship to nuclear receptors in animals, remains unknown. These may favour pathogenesis [36], since fungal steroid synthesis is similar to that in the animal world [4]. Recently, *Achlya* carrier was found complexed with proteins similar to heat shock proteins in mammalian cells [16]. Thus, heat shock protein–receptor association (Fig. 1) appears to have evolved early in systems responsive to steroid hormones.

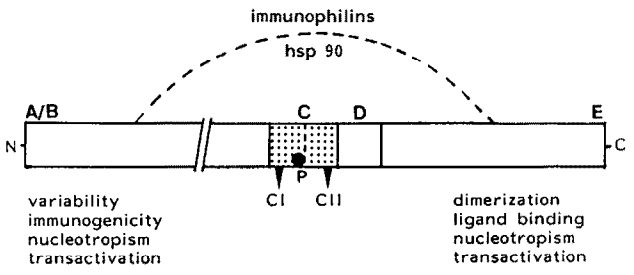

Fig. 1. Schematic representation of receptor mediated activation of transcription by steroid hormones in animals. An intracellular receptor protein can be divided into five major functional domains (A–E). The highly variable, immunogenic, N-terminal A/B domain is rather long in the human mineralocorticoid receptor (984 total residues) but very short in the estrogen receptor (595 total residues). The DNA-binding C domain contains 65–70 amino acids and is highly conserved in the family of 32 proteins cloned to date. It possesses two zinc fingers (CI and CII) for specific recognition of DNA sequences via a 'P' box at the end of CI. A hinge region (D) of 40–50 residues separates C from the carboxy-terminal hormone binding domain (E). The E region consists of 250 residues which are partially conserved. Both the A/B and the E domains contribute to nucleotropism and transactivation. In the resting state, the receptor domains remain occluded by heat shock proteins (HSP 90) and immunophilins of 55–59 kDa, but access to the E domain is left exposed. The binding of a suitable ligand to E initiates activation leading, successively, to the dissociation of occluding proteins, change in receptor conformation, zinc finger mediated receptor dimerization the hormone response elements in the major groove of DNA, and the formation of a preinitiation complex at the proximal promotor (TATA box) of the structural gene for the initiation of mRNA synthesis. Fig. 1 schematically represents the principles of ligand mediated transactivation, and does not constitute an exhaustive review. For further details see [29–31,38] and references therein.

The binding of steroids to receptor-like carriers in photosynthetic plants has been demonstrated only very recently. Cortisol could be bound specifically to a protein in mung bean seedlings but this carrier was not characterized any further [21].

Our studies on the fresh water green alga *Chlamydomonas* have revealed that a 52 kDa cytosolic protein shares close functional and structural kinship with the steroid receptor system in animals. A polyclonal antibody against the 107 kDa rat kidney mineralocorticoid receptor recognizes the 52 kDa alga carrier during immune precipitation and immune displacement assays [22,23].

A number of synthetic derivatives, prepared for specificity to the mammalian mineralocorticoid receptor [29], can be bound to the protein in alga cytosol, and alter *Chlamydomonas* cell multiplication in vitro. R-5020 can also be covalently linked to the carrier in alga by a photochemical procedure, further suggesting structural and functional kinship with the ligand binding domain in animal steroid receptors [22,23].

The presence of a functional DNA-binding domain in the carrier from this alga was assessed by thermal activation. This procedure increases affinity of *Chlamydomonas* protein for DNA cellulose [22,23], in close similarity with the animal steroid receptors (Fig. 1; [29]). Our preliminary studies suggest the presence of a similar protein carrier in vascular plants, such as tobacco and potato (unpublished observations). Hybridization of conserved mammalian receptor DNA sequences with mRNA from plant sources may further confirm the presence of such gene(s) outside the animal kingdom. No other reports regarding the plant world, in this context, are found in the literature.

4. FUTURE PERSPECTIVES

It appears that cell function is influenced by steroids even in the primordial pioneers of life on earth. The biological action of steroids in many plant species appears comparable to that in the animals. Although some of the ancestors left no fossil record, steroids have been constantly present during evolution not only as cell constituents but also as bioregulators [1,2]. In fact, the present day pattern of steroid secretion from specialized glands appears relatively recent in evolutionary terms [1,2].

Increasing complexity in anatomical organization of eukaryotes necessitated reciprocal adjustments in chemical communications for the coordination of intercellular events. Although mammalian steroid receptors are mostly intracellular [14,29–31], increasing evidence suggests that they may also be present in the membrane [26,27]. In fact, soluble receptors in eukaryotes may have arisen from the internalization of membrane receptors of the type found in unicellular organisms, rather than by a chance encounter between intracellular proteins and loitering steroids [37].

Molecular cloning of plant receptors, followed by sequence alignment, may reveal that plants are very much a part of the phylogenetic tree where steroid receptors probably evolved by successive shuffling of a primordial ancestor gene to increase sensitivity of transactivation pathways [38]. Therefore, the principles of zinc finger mediated transcription regulation were probably laid down at the very origin of life processes, and not during the early Cambrian burst of metazoan evolution at arthropod/mammalian dichotomy only 500 million years ago.

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