A Novel Steroid Receptor Elucidates Non-Classical Signal Pathways

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Summary

Cell signaling is a vital mechanism that ensures homeostatic conditions within a biological system. Steroid hormones and their specific receptors play a crucial role in the signaling network. It now appears that a new class of receptor has been isolated, which may finally answer the question of whether a physiologically relevant membrane steroid receptor actually exists.

Between-cell communication is necessary not only for the proper development and differentiation of cells, but it also aids in an organism's ability to respond appropriately to a vast number of stimuli from its internal and external environments. Steroid hormones are key players in this communication system. Upon release from specific glands, steroids locate their receptor inside a target cell, sometimes vast distances away, and bind with high affinity (Falkenstein *et al.*, 2000). Following binding, the receptor is able to affect gene transcription; this, in turn, produces the ultimate response of the biological system (Hammes, 2003). The complexity of this process lends itself to vast forms of regulatory feedback, which manifests itself in a prolonged transduction process that usually takes hours to complete (Hammes, 2003).

However, it has been noted that in some instances, response to steroid hormones occurs much quicker than the above pathway would allow (Zhu *et al.*, 2003). Furthermore, in contrast to the agenda of classical steroid receptors, activation of some steroid receptors generates a response not directly in the form of altered transcriptional activity (Revelli, *et al.*, 1998). These ambiguities challenge our understanding of steroid receptors: how much of the mechanistic scheme involved in their production and signaling have yet to be uncovered?

In March 2003, Zhu and colleagues began to answer this question by revealing that there may indeed be a steroid hormone receptor whose characteristics explain the above ambiguities (Zhu *et al.*, 2003). This receptor, which is specific for progestins and has been isolated from *Cynoscion nebulosus* (spotted seatrout) ovaries, functions at the cell membrane, not intracellularly (Zhu *et al.*, 2003). Furthermore, upon activation, this receptor does not directly interact with DNA to affect gene transcription, but instead sets forth a signal cascade. This allows for a quicker cellular response atypical of classical steroid receptors (Zhu *et al.*, 2003). Previous research in the field has proposed the same argument for other steroid receptors. For example, androgen receptors isolated from male rat osteoblasts seem to act in the same, nongenomic manner (Lieberherr and Gross, 1994), as do the estrogen receptors isolated from pancreatic cells (Nadal *et al.*, 2000). However, the argument of Zhu and colleague is the most enticing, as they are the first to show that their receptor meets all of the criteria for a steroid receptor. This includes structural plausibility, tissue specificity, plasma membrane localization, characteristic steroid binding, activation of a signal transduction pathway, hormonal regulation, and biological relevance (Zhu *et al.*, 2003).

Following cloning of the receptor from a spotted seatrout ovarian cDNA library, computer analysis suggested that the receptor could be a seven-pass transmembrane protein, a plausible and common receptor structure. The specific arrangement of hydrophilic and hydrophobic regions within the protein, which was mapped using hydrophilicity screening, further supports the notion of a transmembrane structure (Zhu *et al.*, 2003).

Zhu and colleagues next expressed the cloned protein *in vivo* to more specifically assess its localization in seatrout tissue (Zhu *et al.*, 2003). Because it was thought that this receptor responds specifically to progesterone, the expectation was that it would be expressed in the gonads, pituitary, and hypothalamus (the sites of progesterone production and secretion) (Zhu *et al.*, 2003). Northern blot analysis confirmed this, as expression of the gene encoding the protein was limited to reproductive tissue and brain tissue (Zhu *et al.*, 2003).

Western blot analysis of ovarian membrane proteins from seatrout oocytes expressing the receptor pinpointed its location even further. If the receptor is indeed a membrane steroid receptor, it is reasonable to assume that it would be localized in the plasma membrane of the cells in which it is expressed. Results of the Western confirmed this reasoning; whereas strong immunoreactivity was observed in the membrane fractions of the seatrout oocytes, no immunoreactivity was detected in the cytosolic fraction (Zhu *et al.*, 2003). Predictably, immunocytochemical analysis verified the results of the Western blot by also showing that strong immunoreactivity was restricted to the oocyte plasma membrane (Zhu *et al.*, 2003).

Zhu and colleagues were able to show that the receptor exhibited characteristic steroid binding through saturation analysis of transfected *E. coli* cells (Zhu *et al.*, 2003). Saturable, high-affinity binding was achieved with physiologically relevant concentrations of progesterone in cells expressing the recombinant protein. This was in contrast to control cells lacking the recombinant protein, where no specific progesterone binding was observed (Zhu *et al.*, 2003). Furthermore, competition binding assays attested to the receptor's reversibility (Zhu *et al.*, 2003).

To determine whether or not the receptor was directly coupled to the activation of a signal transduction pathway, cAMP and MAP kinase analyses were performed on human breast cancer (MDA-MB-231) cells transfected with the protein (Zhu *et al.*, 2003). cAMP analysis showed that progesterone treatment had an inhibitory effect on cAMP production; consequently, cytosolic cAMP concentrations were significantly reduced (Zhu *et al.*, 2003). This effect was not seen upon progesterone treatment of control cells lacking the receptor (Zhu *et al.*, 2003). MAP kinase

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analysis detected a dose-dependent activation of MAP kinase by progesterone in transfected cells, but no activation occurred in control cells lacking the receptor (Zhu *et al.*, 2003). The results of these analyses gave direct evidence that the receptor plays a role in the activation of intracellular signaling pathways. Zhu and colleagues specifically suggest that the receptor is an inhibitory G-protein that regulates a cAMP-dependent cascade (Zhu *et al.*, 2003). This seems especially plausible, considering the fact that the inhibitory effect of progesterone treatment on cAMP production could be reversed by pretreating cells with pertussis toxin, an inhibitor or inhibitory G-proteins (Zhu *et al.*, 2003).

Zhu and colleagues examined whether the progestin receptor was hormonally regulated by incubating ovarian tissues expressing the receptor with 20 β-S, a progesterone metabolite. Since it is characteristic of steroid receptors to be either upregulated or downregulated by the hormone for which they are specific, it was not surprising that dose-dependent upregulation of both mRNA and protein levels following 20 β-S treatment were detected (Zhu *et al.*, 2003). On the other hand, no changes in expression were observed in control tissues incubated without the metabolite (Zhu *et al.*, 2003).

Finally, the biological relevance of this receptor in fish oocytes was addressed by Western blot analysis. The results of the Western showed that the receptor's expression level varies at different stages of oocyte maturation; a rapid increase occurs during the growth of oocytes to full maturity, which is followed by a rapid decline after the oocytes have ovulated (Zhu *et al.*, 2003). This finding led Zhu *et al.* to conclude that the receptor plays a vital role in oocyte maturation (Zhu *et al.*, 2003). Validating this conclusion was the fact that upon injection of zebrafish oocytes with the protein's antisense, oocyte maturation was significantly blocked (Zhu *et al.*, 2003).

In brief, the protein isolated and classified in this experiment is the best case to date for a steroid receptor unrelated to the classical set of intracellular receptors. Even more substantially, this is the first membrane steroid receptor that has been shown to fulfill all of the criteria designated to steroid receptors; its ability to meet all of these criteria has cleared up much of the controversy surrounding whether or not a nongenomic steroid receptor is a physiologically relevant concept. Furthermore, the suggestion that this protein may well be a G-protein coupled receptor is a truly novel idea; if confirmed, it will represent a separate subclass of this receptor type unlike any other that has previously been characterized (Zhu *et al.*, 2003).

Zhu and colleagues made a tremendous contribution to the study of steroid receptors, as their experiments laid the foundation for future areas of research. Since their finding, another research team has been successful in classifying both progesterone and estrogen receptors that function in a similar, nongenomic fashion in *Xenopus* oocytes; these receptors are also thought to play a role in oocyte maturation (Bayaa *et al.*, 2002; Maller, 2001; Tian, 2000). Even more substantially, researchers have began to isolate steroid receptors homologous to the one characterized by Zhu *et al.* in a vast number of vertebrate species, including humans (Zhu, Bond, and Thomas, 2003).

The goal now is to sequence the new-found genes whose products lead to the expression of these receptors and to gain insight into the unique structural resemblances of these receptors to known G-proteins (Zhu *et al.*, 2003; Zhu, Bond, and Thomas, 2003). This includes determining whether the signal molecules and pathways involved in regulating the functional activity of G-proteins are applicable to these newly classified nongenomic steroid hormone receptors. Hopefully, investigation of these issues will provide insight into the elaborate mechanistic schemes by which these receptors operate. This, in turn, will lead to clinical advances, as it will be possible to develop alternative pharmacological approaches for disease prevention and gene therapy (Falkenstein *et al.*, 2000) Clearly, the diverse array of physiological processes steroid hormones are involved in, from conception and fetal development to immune system regulation and the activity of the central nervous system, makes the continuance of research in this field not only inevitable, but crucial.

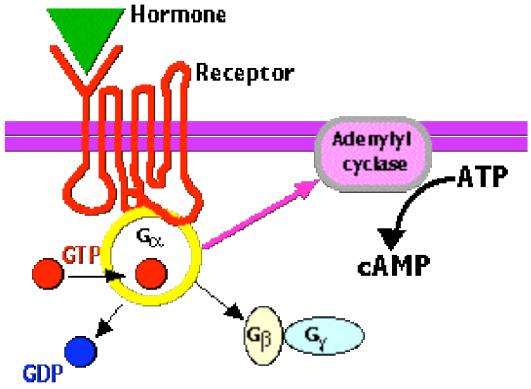

Figure 1. Novel pathway for fish oocyte progesterone receptor.
The binding of progesterone activates the progestin receptor, and a transduction pathway characteristic of G_i proteins occurs; deactivation of adenyl cyclase reduces cytosolic cAMP levels. This change in cAMP concentration, in turn, activates the MAP kinase cascade.

Note: Eukaryon is published by students at Lake Forest College, who are solely responsible for its content. The views expressed in Eukaryon do not necessarily reflect those of the College.

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