

# A perspective for understanding the modes of juvenile hormone action as a lipid signaling system

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## Summary

The juvenile hormones of insects regulate an unusually large diversity of processes during postembryonic development and adult reproduction. It is a long-standing puzzle in insect developmental biology and physiology how one hormone can have such diverse effects. The search for molecular mechanisms of juvenile hormone action has been guided by classical models for hormone–receptor interaction. Yet, despite substantial effort, the search for a juvenile hormone receptor has been frustrating and has yielded limited results. We note here that a number of lipid-soluble signaling molecules in vertebrates, invertebrates and plants show curious similarities to the properties of juvenile hormones of insects. Until now, these signaling molecules have been thought of as uniquely evolved mechanisms that perform specialized regulatory functions in the taxon where they were discovered. We show that this array of lipid signaling molecules share interesting properties and suggest that they constitute a large set of signal control and transduction mechanisms that include, but range far beyond, the classical steroid hormone signaling mechanism. Juvenile hormone is the insect representative of this widespread and diverse system of lipid signaling molecules that regulate protein activity in a variety of ways. We propose a synthetic perspective for understanding juvenile hormone action in light of other lipid signaling systems and suggest that lipid activation of proteins has

evolved to modulate existing signal activation and transduction mechanisms in animals and plants. Since small lipids can be inserted into many different pathways, lipid-activated proteins have evolved to play a great diversity of roles in physiology and development. *BioEssays* 25:994–1001, 2003.

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## Introduction

Perhaps the most striking feature of the juvenile hormone of insects is the extraordinarily broad range of its developmental and physiological effects. Juvenile hormone (JH) plays a role in the control of larval development and metamorphosis and various aspects of adult reproduction, and it also participates in the control of diapause, migratory behavior, wing length polyphenism, seasonal form development, caste determination in Hymenoptera (ants, bees and wasps) and Isoptera (termites), and horn development in scarab beetles, to name just a few.<sup>(1)</sup> It has long been a puzzle for insect physiologists and developmental biologists to understand how it is possible for a single hormone to have such a great diversity of effects. The solution to this puzzle is not helped by the fact that, even after several decades of dedicated work, the mechanism of action of JH is still largely unknown.

The search for a nuclear receptor for JH has been frustrating, in no small part because JH binds to a great many kinds of proteins. Some of these proteins are carriers, others are catabolic enzymes, and yet others, such as the ultraspiracle (USP) component of the ecdysone receptor, suggest an atypical role for JH in the regulation of transcriptional activity. JH also binds with various affinities to a variety of other membrane-bound and some cytoplasmic proteins. This binding is generally considered to be non-specific and, consequently, there has been little interest in exploring these interactions of JH further.<sup>(1,2)</sup>

The failure to obtain experimental evidence for a traditional nuclear receptor mechanism for JH, coupled with the fact that JH interacts with many different proteins, suggests to us that JH does not exert its many and diverse effects through a single conventional hormone signaling mechanism. We thought, therefore, that it might be useful to look for potential mechanisms of JH action among the ways in which other lipid-soluble signaling molecules exert their molecular and physiological effects.

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Abbreviations: ABA, abscisic acid; DIF-1, differentiation-inducing factor-1; FXF, nuclear farnesol receptor; GP, G-protein; GPCR, G-protein-coupled receptor; JH, juvenile hormone; JHBP, juvenile hormone-binding protein; MAPK, mitogen-activated protein kinase; NR, transcriptional regulator; PAL, protein-activating lipid; PDK, phosphoinositide-dependent kinase; PIP<sub>3</sub>, phosphatidylinositol-trisphosphate; PKC, protein kinase-C; PTK, protein tyrosine kinase; R, receptor; RTK, receptor tyrosine kinase; RXR, retinoid X receptor; SR, steroid hormone receptor; USP ultraspiracle protein; 1,25 VD<sub>3</sub>, 1-alpha, 25-dihydroxyvitamin D<sub>3</sub>.

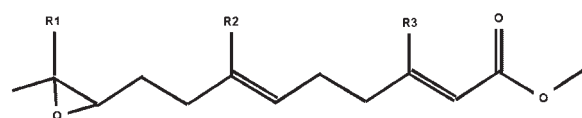
In the sections below, we review the general properties of JH and the mechanisms by which it is believed to exert its cellular effects. We follow this by outlining a series of analogous signaling mechanisms found in organisms, including plants, and conclude that JH may be the insect representative of a diverse and widespread array of lipid signaling molecules. These lipids alter protein activity and may represent a complex of mechanisms that includes, but is not limited to, classical steroids.

### Chemistry of JH

JH has been studied from several perspectives by researchers with interests in basic understanding as well as in developing commercial applications. Soon after the isolation of JH in insects, the agrochemical industry focused its attention on the chemical identity of naturally occurring hormones and on their structure–activity relationships. This work culminated in the design of hormone mimics now used as both arthropod-specific pesticides and research tools. Considerable research has focused on the regulation of juvenile hormone titers through synthesis and breakdown, with an eye to interfering with its *in vivo* regulation. In addition, insect physiologists, concerned with both applied and basic research goals, have worked to understand how JH is involved in the regulation of numerous aspects of insect development and reproduction.

Juvenile hormone is unusual among animal hormones in that it is a sesquiterpenoid, that is, a terpene consisting of three isoprene (5-C) units. Terpenoid molecules and their derivatives often function in communication within and between organisms.<sup>(3)</sup> Sesquiterpenes in particular are widespread in plants and fungi, where they are typically involved in defense against insect herbivores. Plants, in addition to synthesizing a variety of sesquiterpenes including authentic JH-III,<sup>(4)</sup> produce many other chemical compounds that act as JH agonists and antagonists.<sup>(5)</sup>

In the JHs, the sesquiterpene skeleton bears a methyl ester group at C-1 and a 10, 11 epoxide at the other end (Fig. 1). The naturally occurring forms JH 0-III differ from each other in the pattern of additional ethyl or methyl group substitutions at positions R1–3. A variant of JH-III that is characteristic of



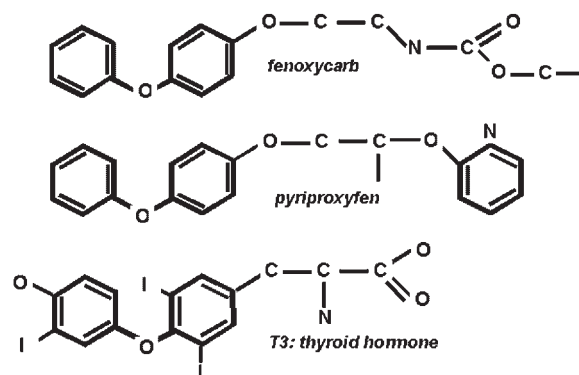
**Figure 1.** JH-III, the most-widespread natural juvenile hormone in insects, has CH<sub>3</sub> groups at R1–3, as shown below. JH-III bisepoxide, found in Diptera, has an epoxide rather than a double bond associated with R2. In other insect JHs, side groups differ as follows: JH-0: R1=R2=R3=C<sub>2</sub>H<sub>5</sub>, JH-I: R1=R2=C<sub>2</sub>H<sub>5</sub> JH-II: R1=C<sub>2</sub>H<sub>5</sub>.

Diptera has a second epoxide group.<sup>(6)</sup> Structure–activity relationships are complex and vary among species.<sup>(7)</sup> Important features required for JH activity include the presence of both the methyl side branches and the terminal carbonyl group.<sup>(8)</sup> The terminal epoxide moiety is not absolutely required, since its replacement with a methyl ether, in the potent mimic methoprene, actually increases activity. Incorporation of aromatic rings at various locations within the carbon chain can also enhance activity, as illustrated by the structures of pyriproxyfen and fenoxycarb, two widely used JH mimics (Fig. 2 and see also Ref. 8). Interestingly, the thyroid hormone triiodothyroxine (T3) is a perfect JH mimic when tested in the JH-mediated volume reduction in the follicle cells of *Locusta migratoria* and *Rhodnius prolixus*, and also shows saturable binding to membrane preparations with K<sub>D</sub> in the low nanomolar range.<sup>(9)</sup> Indeed, the structure of T3 resembles that of certain synthetic JH mimics with two aromatic carbon rings with an ether linkage (Fig. 2).

The fact that many potent mimics of JH, both natural and synthetic, have structures that are quite dissimilar to the natural JHs of insects suggests that specific structure–activity relationships may not be very revealing as to the natural mode of action. General features of solubility, polarity, stability and chain length all contribute to efficacy.<sup>(10)</sup>

### Approaches to studying the mode of JH action

Studies on the mode of action of juvenile hormone have been guided by experience with other animal hormones, particularly those of vertebrates.<sup>(2,11)</sup> The two major classes of vertebrate hormones are the non-polar lipid-soluble steroids and the polar water-soluble peptides. Juvenile hormones belong to neither class, but they have some of the general properties of steroids in their lipid solubility, biosynthetic pathways, and the specificity conferred by side chains.



**Figure 2.** Potent JH mimics that include two aromatic rings connected by an ester linkage.

### *Guided by the steroid model*

The mechanism of action of steroid hormones has been believed to provide the best available model for the mode of action of JH;<sup>(12)</sup> thus, enormous effort has been focused on the identification of intracellular JH receptors. According to the classical model of steroid hormone action, hydrophobic hormones pass through the plasma membrane and bind to a receptor protein in the cytoplasm or nucleus. The hormone–receptor complex acts as a transcriptional regulator that either enhances or represses the expression of specific genes. Guided by the steroid example, JH-mediated transcription has been investigated experimentally by looking for intracellular receptor proteins and transcription factors, as well as by sequencing and analyzing upstream control regions or presumptive target genes in search of diagnostic regulatory domains. Identification of proteins that bind to JH has been greatly facilitated by photoaffinity analogs of synthetic JHs.<sup>(13)</sup> Nevertheless, the isolation of JH receptors has been hindered by the fact that there are many JH-binding proteins (JHBPs) as well as degradative enzymes that bind JH with various affinities. Within this background, it has been difficult to isolate a JH-specific receptor. In general, the non-receptor JH-binding proteins have, or are believed to have, important roles in the distribution, protection and delivery of the hormone to the target cells.<sup>(2)</sup>

*Drosophila melanogaster* has been used as a model organism for studying mechanisms of juvenile hormone action, especially in the search for a nuclear receptor. One candidate receptor protein is the Met (Methoprene-tolerant) protein, which belongs to the bHLH-PAS family of nuclear transcriptional regulators. The protein is found exclusively in nuclei and binds both natural JH-III and the JH mimic, methoprene. Mutations at the *met* locus result in resistance to the disruptive effects of methoprene to which the mutant protein has a reduced ligand-binding affinity.<sup>(14–16)</sup>

Another protein that binds JH, and has been proposed as a JH receptor, is ultraspiracle (USP). USP is the homolog of the vertebrate retinoid X receptor (RXR) and binds JH-III with some specificity (dissociation constant  $<0.5 \mu\text{M}$ , Ref. 17). Methoprene competes with JH-III for binding to USP.<sup>(18)</sup> In a lepidopteran cell line, recombinant USP from *Drosophila melanogaster* homodimerizes and then enhances promoter activation of a transfected heterologous core promoter based on the control region of a gene for juvenile hormone esterase.<sup>(19)</sup> These results indicate that USP can bind a natural JH and subsequently activate the transcription of a gene containing a *cis*-acting region responsive to JH.

The upstream control regions of JH-regulated genes have been investigated in *Locusta migratoria*, where JH stimulates the appearance of a putative transcription factor that induces transcription of the *jhp21* gene coordinately with vitellogenin (yolk protein). The upstream region of *jhp21* contains three copies of an apparent JH response element, a partially palin-

dromic 15-nt sequence. Interestingly, the transcription factor that recognizes this element does not appear until 24 hours after methoprene treatment,<sup>(20,21)</sup> suggesting that it may not play a role in an early response.

### *Alternative modes of steroid hormone action*

Although steroid hormones act by regulating gene transcription, it has been recognized for some time that steroids can elicit cellular responses that are rapid and inconsistent with a mechanism that depends on the typically slower effect on transcription.<sup>(22)</sup> In the past decade, a number of steroid and steroid-like hormones have been found to have receptors and action at the cell membrane. Molecular mechanisms underlying cell surface transduction (sometimes also referred to as non-genomic signal transduction) include pathways that act via protein kinase C (PKC), protein kinase A, intracellular calcium release, intracellular pH, and mitogen-activated protein kinases (MAPK). It is now clear that at least some steroids can have both genomic and cell surface targets.<sup>(23)</sup>

Examples of steroid signaling molecules that have cell-membrane receptors, in addition to classical nuclear ones, include progestin, estrogen and ouabain. In sea trout, a progestin receptor has been identified that is found only in the plasma membrane of reproductive tissue. The protein is a G-protein-coupled receptor that fulfills all criteria for a true steroid receptor.<sup>(24)</sup> Estrogen acts through receptors in the cell membrane or cytosol and through second messenger signaling, and these effects are particularly well studied in the central nervous system.<sup>(25)</sup> In hamster ovaries, both cell membrane and nuclear estrogen receptors originate from a single transcript. The membrane receptors, when bound, activate G proteins and stimulate inositol phosphate production and adenylate cyclase activity.<sup>(26)</sup> Effects through this receptor are transduced via the activation of G proteins as well as tyrosine kinase signaling pathways. In mammalian renal tissue, the binding of membrane receptors by the steroid ouabain induces regular, low-frequency intracellular calcium oscillations that activate the transcription factor NF-KB.<sup>(27)</sup>

### *JH and the alternative steroid model*

A number of lines of evidence suggest that some actions of JH in insects occur via membrane receptors and a PKC signaling pathway. PKCs form a family of biochemically similar kinases that play key roles in the regulation of growth, differentiation, and carcinogenesis. PKCs are activated by the second-messenger diacylglycerol and phosphorylate other cytoplasmic and membrane proteins.<sup>(28)</sup>

Systems in which the response to JH is dependent on PKC signaling include male accessory glands in *D. melanogaster* and ovarian follicle cells in *Rhodnius prolixus* and *Locusta migratoria*. In *Drosophila*, the male accessory glands make several proteins following copulation. This response can

be mimicked *in vitro* by exposing virgin glands to JH-III, in the presence of sufficient calcium ions. Glands from flies with mutant PKC are unable to respond.<sup>(29)</sup> In *R. prolixus* and *L. migratoria* females, JH induces ovarian follicle cells to shrink, creating spaces between them through which yolk proteins can move rapidly into developing oocytes. In *Rhodnius*, the response to JH-I involves PKC signaling and requires a Na<sup>+</sup>/K<sup>+</sup> ATPase. *Locusta migratoria* appears to have a similar system, using JH-III rather than JH-I.<sup>(1)</sup>

Sesquiterpenoid signaling via PKC may be widely distributed among the invertebrate phyla. Methyl farnesoate, for instance, is the functional equivalent of juvenile hormone in Crustacea, and in the barnacle *Balanus amphitrite*, both JH-III and methyl farnesoate induce metamorphosis via PKC activation. Metamorphosis of the annelid *Capitella* can be induced by either methylfarnesoate or JH-III, an effect that likewise appears to be mediated by PKC activation.<sup>(30,31)</sup>

The response of both the male accessory gland of *Drosophila* to JH-III and barnacle larvae to methyl farnesoate can be mimicked by phorbol esters.<sup>(29,30)</sup> Phorbol esters are polycyclic diterpenes that have been studied extensively for their tumor-promoting properties. They, like phytomimics of JH, are found in plants where they can function as insect deterrents.<sup>(3)</sup>

#### Other signaling systems with some similarity to juvenile hormone and its action

Throughout the animal and plant kingdoms there are instances of hormone-like signaling systems that have curious similarities to JH. They share with JH the properties that a great diversity of biological effects are exerted through a number of different, apparently unrelated, molecular mechanisms. Individually, these signaling systems may appear to be idiosyncratic phenomena, each restricted to a small group of taxa. Taken together, they show that a variety of lipid-soluble molecules exert powerful effects on physiology, gene expression and development, without acting directly in gene transcription. Lipid-soluble vitamins, prostaglandins and isoprenoids are all well characterized in, but not restricted to, vertebrates. Abscisic acid (ABA) in plants is a sesquiterpenoid phytohormone with a broad diversity of roles in the transitions from embryonic to germinative growth and from vegetative to reproductive growth. DIF-1 in *Dictyostelium* is a lipophilic molecule that mediates the determination of stalk cells versus reproductive spores.

#### Lipid soluble vitamins

Lipid vitamins have functions resembling those of hormones, and some even act through nuclear receptors belonging to the steroid receptor superfamily. Vitamin A and vitamin D are lipid vitamins that are precursors for the active signaling molecules all-*trans* retinoic acid and 1- $\alpha$ , 25-dihydroxyvitamin D<sub>3</sub> (1,25 VD<sub>3</sub>). Their nuclear receptors are termed the retinoic

acid receptor and the vitamin D receptor, respectively. Although 1,25 VD<sub>3</sub> is not a steroid, it is synthesized from a steroid precursor and resembles JH in that it is linear and flexible. Two different receptors recognize different configurations. In its bowl shape, 1,25 VD<sub>3</sub> interacts with a nuclear receptor and, in its alternative planar shape, it is recognized by a putative membrane receptor.<sup>(32)</sup> In mammalian kidneys, 1,25 VD<sub>3</sub> upregulates P450-C24 activity through both genomic and nongenomic responses. The slower genomic response involves binding of 1,25 VD<sub>3</sub> to a nuclear vitamin D receptor. The hormone–receptor complex interacts with retinoid-x-receptor and associated coactivators to increase the rate of transcription. The rapid response through membrane receptors involves PKC and MAPK, which phosphorylate transcription factors.<sup>(33)</sup>

In contrast to vitamins A and D, the chemically similar vitamin E is an active signaling molecule in its own right, and is not known to have nuclear receptors<sup>(34)</sup>. The most-active form of vitamin E is alpha-tocopherol. In vertebrates, alpha-tocopherol inhibits the growth of vascular smooth muscle cells apparently by inhibiting PKC activity.<sup>(35)</sup> In rotifers, alpha-tocopherol regulates female polymorphisms and sexual reproduction.<sup>(36)</sup> In this respect, alpha-tocopherol has a biological function that is very similar to that of JH in the control of insect polyphenism and reproduction.<sup>(37)</sup>

#### Prostaglandins

Prostaglandins are derived from arachidonic acid, and belong to a class of lipids called eicosanoids (having chains of 20 carbons). In vertebrates, prostaglandins are involved in autocrine/paracrine signaling loops in many tissues. They are key mediators of inflammation and other responses to injury, and also act as signals for the contraction of smooth muscle (e.g., of uterus, blood vessels, intestines), the control of gastric secretion, and blood platelet aggregation. Prostaglandins also occur in insects and many other invertebrates.<sup>(38,39)</sup> In insects, they appear to be mainly involved in regulating the cellular immunological defense mechanisms and have been shown to be involved in the regulation of oviposition.

Consistent with the pattern emerging for other lipid signaling molecules, including JH, prostaglandins appear to exert their effects through several different mechanisms. Prostaglandins act primarily by binding to G-protein-coupled cell surface receptors and exert their effect through Ca<sup>2+</sup> and cAMP second messenger signaling. There is some evidence, however, that some prostaglandin responses are elicited by binding to nuclear proteins that interact with one of the nuclear receptors for retinoic acid.<sup>(40,41)</sup>

#### Prenylation

Post-translation modification of regulatory proteins by binding to lipids can have profound effects on their biological activity. Terpenoid modifications of proteins have been found to play

important roles in diverse signalling pathways and regulatory mechanisms in plants, fungi and animals. One important type of modification that is apparently important in all organisms is prenylation, in which a farnesyl or geranylgeranyl is covalently bound to the protein's carboxy terminal. The result is that the bound proteins are brought into position to interact with other membrane-bound molecules. Prenylation was first discovered in fungi, when it was shown that their mating pheromones contained a farnesyl moiety.<sup>(42)</sup> Prenylation is achieved by specialized enzymes, prenyltransferases, which attach either a farnesyldiphosphate or geranylgeranyldiphosphate to a cysteine near a protein's carboxy terminal.<sup>(43)</sup> No deprenylating enzymes have been described that cleave the prenyl group from intact proteins.<sup>(64)</sup>

Many membrane-directed proteins are prenylated,<sup>(44,45)</sup> and prenylation has been best studied in the Ras proteins of vertebrates. Ras proteins are small GTPases that are active when bound to GTP and inactive when bound to GDP. They must be prenylated to be anchored on the membrane before they are able to respond to signals from receptor tyrosine kinases (RTKs). In response to these signals, Ras acts on Raf and initiates the MAPK signaling cascade that results in phosphorylation of various proteins including nuclear transcription factors.<sup>(46)</sup>

Prenylation is perhaps best characterized in vertebrates, but it also has received considerable attention in yeast and plants.<sup>(47,48)</sup> Prenyl transferases have been characterized in insects as key enzymes in juvenile hormone biosynthesis.<sup>(49)</sup> However, potential roles for more general protein prenylation have not been explored in insects. Since they are derived from farnesyl, it is possible that juvenile hormones could exert some of their effects by prenylating signaling proteins.

### *Abscisic acid*

Abscisic acid (ABA) in plants may provide another heuristic model for juvenile hormone action. ABA is a sesquiterpenoid phytohormone with a broad diversity of roles in the control of seed dormancy, seed maturation and germination including the synthesis of storage proteins and lipids, and the transitions from embryonic to germinative growth and from vegetative to reproductive growth.<sup>(50)</sup> ABA is also involved in rapid responses such as the environmentally induced closure of stomatal guard cells. Interestingly, in analogy to JH's role in regulating insect polyphenisms, ABA is known to play a role in the induction of heterophylly, the alternative forms of leaves that develop in different environments in some plants<sup>(51)</sup>

As with JH, the search for a nuclear receptor of ABA has been fraught with frustration. No receptor has been definitively identified yet, and the fact that some responses to ABA are very rapid and others are delayed, suggests that this hormone may induce its effects via several different mechanisms.<sup>(50)</sup> ABA response elements have recently been characterized,

and the promoter regions of some genes contain multiple elements that may cooperate in induction.<sup>(52)</sup> Genomic analysis has shown that the expression of over 84% of the 1354 ABA responsive genes was dramatically changed by a mutation of one particular protein phosphatase 2C gene (ABI1), showing that a substantial portion of ABA responsiveness is altered if this phosphorylating enzyme has a particular defect.<sup>(53)</sup> The importance of ABI1, together with the observation that ABI1 and ABI2 also interact with PKS3, another protein kinase,<sup>(54)</sup> suggests that a large proportion of ABA effects may be mediated by a common phosphorylation mechanism.

Also, it is interesting to note that the transduction of ABA signals in the control of seed dormancy in *Arabidopsis thaliana* is mediated by a prenylation mechanism. A mutation that increases a seed's sensitivity to ABA codes for a subunit of a farnesyl transferase. Decreased farnesyl transferase activity reduces the farnesylation of calmodulin, a negative regulator of ABA activity. As a result, calmodulin does not become anchored to the membrane, but is instead translocated to the nucleus.<sup>(55)</sup>

It seems that plants and animals present us with several independently evolved signaling systems with similar functions, and the comparative study of these systems may be generally useful in the elucidation of general principles. In addition to the similarities of ABA and JH, there are substantial similarities between auxins and serotonin, ethylene and nitrous oxide, and between jasmonic acid and prostaglandins.<sup>(56)</sup>

### *DIF-1 in slime molds*

Differentiation-inducing factor-1 (DIF-1) is an example of a lipid-soluble molecule that is important in determining major changes in gene transcription in a protist. For *Dictyostelium amoebae*, extracellular cAMP is the principal aggregation signal, which acts together with another secreted molecule, DIF-1, to control differentiation of the stalk cells.<sup>(57,58)</sup> DIF-1 is a phenyl hexanone and is unusual among lipid-based signaling molecules in that it is chlorinated. DIF-1 appears to exert its effect by increasing the transcription of protein kinase genes but the pathway by which this stimulation occurs is still unknown.

DIF-1 illustrates the large degree of cross-system reactivity that is common among lipid-signaling molecules. DIF-1 has been shown to have biological effects in cultured human leukemia cells, where it inhibits growth by arresting cells in the G<sub>1</sub> phase. In addition, DIF-1 induces apoptosis in rat pancreatic cells and induces erythroid differentiation in another leukemia cell line.<sup>(59)</sup> In these cells, DIF-1 increases the intracellular calcium ion concentration by release from intracellular stores, and in doing so promotes retinoic acid-induced cell differentiation. Evidently DIF-1 is able to mimic some vertebrate signaling factors.

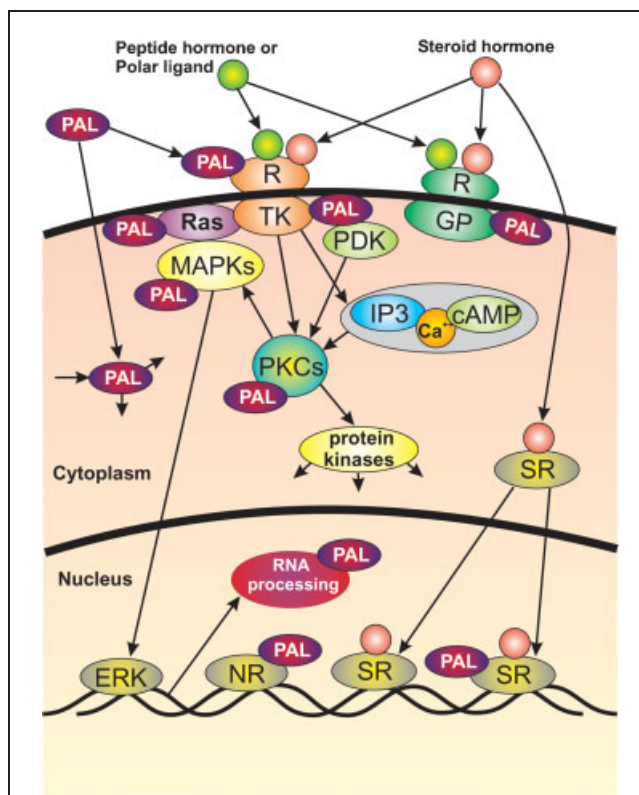
### Concluding remarks

In eukaryotic organisms, a wide diversity of signaling systems are based on lipid and lipophilic molecules (Fig. 3). Mechanisms of action vary, and the diversity of these mechanisms has made it difficult to recognize common patterns among them. Steroids typically act through classical nuclear receptors and thus directly affect gene transcription. But steroids can also have alternative modes of action that are similar to those of other signaling molecules that act through membrane receptors. Signaling pathways initiated by way of membrane receptors include activation of RTK and GPCR, which activate second messengers and PKC. Depending on the specific pathway, PKC can interact with MAPK and a variety of protein kinases, some of which are transcription factors. Indeed, it appears that classical steroid signaling is but one specialized case of a signal transduction pathway used by lipid signaling molecules.

We suggest that the juvenile hormones are members of a diverse and widespread class of lipid signaling molecules that can participate in both signal transduction and transcriptional regulation. In view of the great diversity of lipid-based signaling systems, it is not surprising that the juvenile hormones can have modes of action that are initiated through proteins associated with the plasma membrane, as well as having a direct interaction with transcriptional regulators in the nucleus. Also, it has been proposed recently that terpenoid-based compounds are signalling molecules that arose in an ancient RNA world and have the potential to act on mRNA directly.<sup>(60)</sup>

Particular features of these lipid signaling molecules may dispose them toward one mode of action or another. An understanding of the advantages of each type of mechanism can be obtained by comparative studies of signaling molecules, such as steroids, that act through both types of mechanisms. The estrogen receptor, for instance, appears to be expressed both as a plasma membrane pool and a nuclear pool that act through kinase signaling and transcriptional transactivation, respectively.<sup>(61)</sup> One possibility is that kinase signaling can speed up or amplify the onset of the transcription response, whereas the nuclear receptor is responsible for maintaining a more sustained response.<sup>(61)</sup> In addition, signaling cascades can be concatenated in various ways, forming networks that are responsive to both peptide and lipid-based signaling molecules. Such networks have also been described or suggested in a wide variety of organisms.<sup>(56,62,63)</sup>

The binding affinities of lipid signaling molecules to their presumptive receptors are often viewed as unusually low. Gilbert et al.<sup>(2)</sup> have noted that various ligands bind to the mammalian nuclear farnesol receptor, FXF, with a  $K_D$  in the micromolar range, rather than in the nanomolar range that is typical for classical hormones. The  $K_D$  of retinoic acid:receptor binding, is also in the micromolar range, as is the  $K_D$  of JH:USP binding. These low binding affinities suggest that the activation



**Figure 3.** Synthetic scheme for the sites of action of protein-activating lipids (PAL). This scheme summarizes the many locations where PALs have been shown to have an effect in various organisms and cell types. The figure shows that PALs can have an extracellular origin (as hormones) as well as an intracellular origin (e.g., prenylation agents). We also include PIP<sub>3</sub>-PDK signaling as another variant of lipid signaling. For clarity, we separate the effects of PALs and steroid hormones, although it is likely that steroid hormones are specialized PALs (see text). Polar ligands and certain steroids, such as a progestin and estrogen, bind to cell surface receptor tyrosine kinases (R + TK) or G-protein-coupled receptors (R + GP). These receptors activate either Ras-Mitogen-Activated Protein Kinase (Ras-MAPK) cascades or second messenger systems (here abbreviated by the IP<sub>3</sub>/Ca<sup>2+</sup>/cAMP cluster), and both can use Protein Kinase C (PKC) as an intermediary. Both Ras and G proteins can be activated and anchored to the cell membrane via PAL modification, and the activities of PKC and certain MAPKs can be modified by PALs. PKCs and MAPKs have their cellular effects either by activating other protein kinases or by transcriptional regulation via molecules like ERK (one of the terminal MAPKs). Steroid hormones can also bind to intracellular steroid receptors (SR). The hormone-receptor complexes then are translocated to the nucleus and act as transcriptional regulators. The activity (and probably the targeting) of these hormone-receptor complexes can be altered by PALs. PALs can also directly activate transcriptional regulators (NR), as in the case of JH activation of USP.

achieved by these lipids is easily reversible and can thus be modulated by moderate changes in concentration of the ligand. Effective signaling may not require the high-affinity binding characteristic of nuclear receptors. Although most lipid modifications are readily reversible, prenylation is an example of a highly evolved variation of a lipid signaling mechanism in which the lipid becomes covalently bound to the protein.

We believe that JH is the insect representative of the widespread and highly diverse lipid–protein activation mechanisms reviewed in this paper. If this synthetic view of the roles of lipids in biological signaling mechanisms is correct, it suggests that the search for classical hormone receptor mechanisms for JH is likely to provide only a limited part of the story. Instead, to understand the many roles of JH it will be useful to investigate the functions of proteins to which JH binds with moderate affinity. Insects are the most diverse of the world's metazoan taxa and display an exuberant amount of polyphenic variation, which is determined, in many cases, by juvenile hormone and fine-tuned by its metabolic regulation.<sup>(37)</sup> The evolution of this remarkable diversity of JH effects may be due in large part to the ability of juvenile hormones to interact with many different types of proteins in a variety of signal transduction and transcriptional activating mechanisms.

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