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Commentary

New ways and new hopes for IGR development

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Insect Growth Regulators (IGRs) represent advanced, bio-rational insecticides. This Special Issue reflects progress in IGR development that has been enabled by insight into the molecular principles of biosynthetic or hormone signaling pathways. The unifying principle is aiming at processes and molecular targets that are unique to arthropods and ideally to narrower insect taxa representing pests or disease vectors. While some strategies of obtaining the desired compounds for chemical intervention rely on rational, structure-based design or computational power, others exploit technologies allowing automated, high-throughput screening of large chemical libraries. All avenues leading to selective and environmentally safe pest control are valid as we face the imminent threat of the declining world insect population.

Keywords: molting, metamorphosis, ecdysone, juvenile hormone, biosynthesis, receptors.

Introduction

Just when conceiving this Commentary, I received an alert from the *Proceedings of the National Academy of Sciences of the USA*, announcing a release on January 11 of their Special Issue on the global decline in insect abundance and species diversity, including the threat to essential pollinators. The Introduction entitled "Insect decline in the Anthropocene: Death by a thousand cuts" explains that while the effects of environmental stressors including climate change, destruction of habitats, and chemical pollution combine, their relative contributions are hard to estimate.[1)] However, the overuse of neonicotinoids and other wide-spectrum pesticides is certainly one of the contributing stressors. Therefore, environmentally friendly insecticides, ideally ones that are harmless to non-target species, may (again) be in demand. The IGRs such as disruptors of insect-specific endocrine and biosynthetic processes hold new promises.

The reviews and original research featured in this Special Issue show examples of new efforts to find compounds with the desired properties through rational, structure-based or computational design, as well as high-throughput screening (HTS) strategies. For my lack of expertise on chitin metabolism, covered by three contributions to this issue,[2–4)] I will focus on those articles concerning endocrine signaling, either at the level of the juvenile hormone (JH) or ecdysteroid biosynthesis, transport, and receptor-mediated downstream effects. A large part of the progress presented in this Special Issue has relied on the atomic-level structure resolution of the EcR-Usp ecdysone receptor complex,[5–7)] and on the identification of the Methoprene-tolerant (Met) protein as an intracellular receptor for JH.[8–10)]

1. Hormone biosynthesis and transport

Picard and colleagues[11)] review the grounds establishing farnesyl diphosphate synthase (FPPS) as a promising target of chemical intervention with lepidopteran pests. Being part of the mevalonate pathway, FPPS contributes to biosynthesis of many essential terpenoids, which in insects include JH. FPPSs occur in all organisms. However, lepidopteran FPPSs display unique structural features, relating to the fact that unlike other insects possessing methyl-branched JH III, moths synthesize ethyl-branched JH homologs (such as JH I)[12)] from bulkier substrates. Data suggest that the unique structural features of lepidopteran FPPSs enable design of selective inhibitors not affecting other species. The ongoing target-specific inhibitor design is aided by ample X-ray structural information on FPPSs, including those from Lepidoptera.[13)]

The team of Ryusuke Niwa presents high-quality original research[14)] leading to inhibitors of a steroidogenic glutathione *S*-transferase that they had previously discovered as a product of the essential *Drosophila noppera-bo* (*Nobo*) gene.[15)] A HTS of a chemical library yielded five non-steroidal Nobo inhibitors acting at micromolar concentrations. Nobo was crystallized with each of the five compounds, revealing their distinct interactions with the enzyme. As Nobo has only been found in dipteran and lepidopteran insects, the identified compounds have a great po-

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tential to be developed into selective IGRs.

An entirely new level of potential intervention with ecdysteroid signaling is discussed in a review by Okamoto and Yamanaka.[16] The idea is based on their discovery of an ecdysone importer (EcI)[17] that is required in the target tissues to uptake the hormone and in the blood-brain barrier to deliver it to the CNS.[18] Loss of EcI corresponds to ecdysteroid deficiency in *Drosophila* mutants. Besides altering the traditional tenet that steroid hormones cross the cell membranes *via* simple diffusion, this transmembrane protein offers a target for developing an arthropod-specific EcI blocker as a new type of IGR. The authors speculate that a prospective ecdysone antagonist acting at the cell-surface level of EcI would be less prone to intracellular metabolism and other forms of resistance.

2. Ecdysone receptor agonists

Three-dimensional structure resolution has been the driving force to elucidate the interaction of non-steroidal ecdysone receptor agonists with the EcR-Usp receptor complex.[19] The group led by Isabelle Billas presents receptor-ligand crystal structures explaining how dibenzoylhydrazine and imidazole-substituted EcR agonists utilize part of the ligand-binding pocket that is occupied with water molecules when binding the natural, active ecdysteroid agonist (20-hydroxyecdysone; 20E). The synthetic compounds cause a major conformational change to the EcR ligand-binding domain, illustrating its flexibility and adaptability to the disparate chemistries.

Ueno and colleagues[20] present a solid study on non-steroidal EcR agonists of the tetrahydroquinoline (THQ) type that are interesting for their target preference of mosquito larvae. The authors have expanded the repertoire of existing THQ compounds through *de novo* synthesis and tested all 35 compounds for receptor binding in a mosquito cell line and in bioassays. The data positively correlate the agonists by their binding affinity, increasing hydrophobicity, and larvicidal potency in *Aedes albopictus*.

3. Cell-based reporter systems and high-throughput search for juvenoid IGRs

Cell-based reporter assays available for studies of both 20E and JH response are extensively reviewed by Ito-Harashima and Yagi.[21] Various systems have been developed thus far to monitor 20E and JH receptor activities through the endogenous hormone receptors in insect cell lines. Alternative, heterologous mammalian or yeast cells expressing the recombinant insect receptor proteins have the advantage of eliminating endogenous target molecules. Most of the reporter assays measure the transcriptional activation of a reporter driven by ecdysteroid-response (EcRE) or JH-response (JHRE) DNA elements derived from known hormone-inducible genes. Two-hybrid assays report interaction between the dimerizing receptor subunits, either EcR and Usp for the ecdysteroid nuclear receptor or Met/Gce and Tai (aka SRC) for the complex of the bHLH-PAS JH receptor proteins. The authors explain how each reporter assay can aid discovery of new IGRs, as exemplified by the non-steroidal agonists of EcR. The potential of cell-based assays for HTS approaches is emphasized. Indeed, initial attempts to identify both inhibitors[22,23] and novel activators (this issue)[24] of JH signaling have been reported.

In their contribution, Kayukawa and colleagues[24] exploit a JHRE-luciferase reporter assay based on a cell line from *Bombyx mori* to screen a large chemical library. The same HTS performed in an antagonistic mode has recently yielded an inhibitor of JH signaling, capable of downregulating the JH-response gene *Kr-h1* and inducing precocious pupation in the silkworm larvae.[23] Here, 10 novel activators of the JHRE reporter are presented, of which several share the 4-phenoxyphenoxymethyl structure of some existing juvenoid IGRs such as pyriproxyfen, while others have distinct chemistry,[24] consistent with the great diversity of JH receptor agonists.[25,26] When tested in final-instar *Bombyx* larvae, seven of the activators could delay metamorphosis although they were unable to prevent pupation and provoke an extra larval molt. Still, the obtained HTS hits could be promising lead compounds for further IGR development.

The article by Yokoi and colleagues[27] sets the first example of a JH receptor agonist discovered through an in-silico approach. The virtual screening of five million compounds relied on a structural homology model of the *Drosophila* JH receptor protein Met. Compounds were computationally selected based on similarity to two potent juvenoid IGRs, fenoxycarb and pyriproxyfen, both agonist ligands of the JH receptors.[8,25,26] In the following virtual screens, the hits were docked to the molecular model and the free energy of the ligand-receptor binding was calculated, finally yielding 11 candidates. Importantly, selected compounds were validated in a JHRE-based reporter assay in a human cell line expressing the *Drosophila* JH receptor proteins Met and Tai.[28] One hit proved to activate the reporter with a sub-micromolar potency, providing a lead compound for further development. Interestingly, this piperazin-based compound is predicted to form a hydrogen bond with a conserved tyrosine residue, previously implicated in forming a hydrogen bond with the epoxide moiety of JH III within the ligand-binding pocket of the other *Drosophila* JH receptor, Gce.[25]

4. Stage-specific organismal effects of JH, juvenoid IGRs, and of a novel insecticide

Although the effect of JH on insect embryogenesis has far-reaching evolutionary implications,[29] it remains subject to ongoing debate.[30] Particularly embryos of hemimetabolous species are considered sensitive to ectopic JH treatments.[29,31] However, this notion relies on defects inflicted by high doses of synthetic JH mimics or JH homologs foreign to the treated species. Naruse and colleagues[32] subject the eggs of the bean bug, *Riptortus pedestris*, to the natural heteropteran JH, identified in this species as JH III skipped bisepoxide ($JHSB_3$).[33] They show that $JHSB_3$ induced developmental malformations and arrest to the bean bug embryos at micromolar doses, whereas the common JH III or juvenoid IGRs such as pyriproxyfen had to be applied in doses at least thousand-fold higher.

Parthasarathy and Palli[34] compile reported effects of commonly deployed JH mimicking insecticides (methoprene, hydroprene, and pyriproxyfen) on survival of three target species when treated at different stages. They emphasize the limited utility and rather partial success of current juvenoid IGRs in insect control. Their review in fact underscores the need of developing better means of intervention at the level of JH signaling.

Satoh and colleagues[35] describe multistep chemical derivatization of existing insecticidal and lead compounds using pyrimidin substituents. Their synthesis yielded benzpyrimoxan, a new, currently registered insecticide active against the brown planthopper (*Nilaparvata lugens*), a major pest of rice. Low toxicity to pollinators and other beneficial arthropods is an advantage of this putative novel IGR. While the planthopper nymphs die during molting, the mode of action of benzpyrimoxan is unknown and its molecular target has not been reported.

References

1) D. L. Wagner, E. M. Grames, M. L. Forister, M. R. Berenbaum and D. Stopak: Insect decline in the Anthropocene: Death by a thousand cuts. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2023989118 (2021).
2) Y. Li, L. Liu, J. Yang and Q. Yang: An overall look at insect chitin deacetylases: Promising molecular targets for developing green pesticides. *J. Pestic. Sci.*, **46**, 43–52 (2021).
3) Y. Mushiake, A. Tsuchida, A. Yamada, H. Kanzaki, T. Okuda and T. Nitoda: New analogs of pochonicine, a potent *β-N*-acetylglucosaminidase inhibitor from fungus *Pochonia suchlasporia* var. *suchlasporia* TAMA 87. *J. Pestic. Sci.*, **46**, 115–119 (2021).
4) K. Mori, H. Tokuoka, H. Miyagawa and Y. Nakagawa: Isoxaben analogs inhibit chitin synthesis in the cultured integument of the rice stem borer (*Chilo suppressalis*). *J. Pestic. Sci.*, **46**, 120–123 (2021).
5) I. M. L. Billas, T. Iwema, J. M. Garnier, A. Mitschler, N. Rochel and D. Moras: Structural adaptability in the ligand-binding pocket of the ecdysone hormone receptor. *Nature* **426**, 91–96 (2003).
6) J. A. Carmichael, M. C. Lawrence, L. D. Graham, P. A. Pilling, V. C. Epa, L. Noyce, G. Lovrecz, D. A. Winkler, A. Pawlak-Skrzecz, R. E. Eaton, G. N. Hannan and R. J. Hill: The X-ray structure of a hemipteran ecdysone receptor ligand-binding domain: Comparison with a lepidopteran ecdysone receptor ligand-binding domain and implications for insecticide design. *J. Biol. Chem.* **280**, 22258–22269 (2005).
7) R. J. Hill, I. M. L. Billas, F. Bonneton, L. D. Graham and M. C. Lawrence: Ecdysone receptors: From the Ashburner model to structural biology. *Annu. Rev. Entomol.* **58**, 251–271 (2013).
8) J.-P. Charles, T. Iwema, V. C. Epa, K. Takaki, J. Rynes and M. Jindra: Ligand binding properties of a juvenile hormone receptor, Methoprene-tolerant. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 21128–21133 (2011).
9) T. Kayukawa, C. Minakuchi, T. Namiki, T. Togawa, M. Yoshiyama, M. Kamimura, K. Mita, S. Imanishi, M. Kiuchi, Y. Ishikawa and T. Shinoda: Transcriptional regulation of juvenile hormone-1 mediated induction of Krüppel homolog 1, a repressor of insect metamorphosis. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 11729–11734 (2012).
10) M. Jindra, X. Bellés and T. Shinoda: Molecular basis of juvenile hormone signaling. *Curr. Opin. Insect Sci.* **11**, 39–46 (2015).
11) M.-È. Picard, M. Cusson, S. S. Sen and R. Shi: Rational design of Lepidoptera-specific insecticidal inhibitors targeting farnesyl diphosphate synthase, a key enzyme of the juvenile hormone biosynthetic pathway. *J. Pestic. Sci.*, **46**, 7–15 (2021).
12) W. G. Goodman and M. Cusson: The juvenile hormones. In "Insect Endocrinology," ed. by L. I. Gilbert, Elsevier, Amsterdam, pp. 310–365 (2012).
13) M.-È. Picard, A. Nisole, C. Béliveau, S. E. Sen, A. Barbar, R. Shi and M. Cusson: Structural characterization of a lepidopteran type-II farnesyl diphosphate synthase from the spruce budworm, *Choristoneura fumiferana*: Implications for inhibitor design. *Insect Biochem. Mol. Biol.* **92**, 84–92 (2018).
14) K. Koiwai, K. Morohashi, K. Inaba, K. Ebihara, H. Kojima, T. Okabe, R. Yoshino, T. Hirokawa, T. Nampo, Y. Fujikawa, H. Inoue, F. Yumoto, T. Senda and R. Niwa: Non-steroidal inhibitors of *Drosophila melanogaster* steroidogenic glutathione *S*-transferase Noppera-bo. *J. Pestic. Sci.*, **46**, 75–87 (2021).
15) S. Enya, T. Ameku, F. Igarashi, M. Iga, H. Kataoka, T. Shinoda and R. Niwa: A Halloween gene *noppera-bo* encodes a glutathione *S*-transferase essential for ecdysteroid biosynthesis *via* regulating the behaviour of cholesterol in *Drosophila*. *Sci. Rep.* **4**, 6586 (2014).
16) N. Okamoto and N. Yamanaka: Transporter-mediated ecdysteroid trafficking across cell membranes: a novel target for insect growth regulators. *J. Pestic. Sci.*, **46**, 23–28 (2021).
17) N. Okamoto, R. Viswanatha, R. Bittar, Z. Li, S. Haga-Yamanaka, N. Perrimon and N. Yamanaka: A membrane transporter is required for steroid hormone uptake in *Drosophila*. *Dev. Cell* **47**, 294–305 (2018).
18) N. Okamoto and N. Yamanaka: Steroid hormone entry into the brain requires a membrane transporter in *Drosophila*. *Curr. Biol.* **30**, 359–366 (2020).
19) C. Browning, A. G. McEwen, K. Mori, T. Yokoi, D. Moras, Y. Nakagawa and I. M. L. Billas: Nonsteroidal ecdysone receptor agonists use a water channel for binding to the ecdysone receptor complex EcR/USP. *J. Pestic. Sci.*, **46**, 88–100 (2021).
20) M. Ueno, T. Yokoi, Y. Nakagawa and H. Miyagawa: Receptor-binding affinity and larvicidal activity of tetrahydroquinoline-type ecdysone agonists against *Aedes albopictus*. *J. Pestic. Sci.*, **46**, 101–108 (2021).
21) S. Ito-Harashima and T. Yagi: Reporter gene assays for screening and identification of novel molting hormone- and juvenile hormone-like chemicals. *J. Pestic. Sci.*, **46**, 29–42 (2021).
22) S.-H. Lee, H.-W. Oh, Y. Fang, S.-B. An, D.-S. Park, H.-H. Song, S.-R. Oh, S.-Y. Kim, S. Kim, N. Kim, A. S. Raikhel, Y. H. Je and S. W. Shin: Identification of plant compounds that disrupt the insect juvenile hormone receptor complex. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 1733–1738 (2015).
23) T. Kayukawa, K. Furuta, K. Nagamine, T. Shinoda, K. Yonesu and T. Okabe: Identification of a juvenile-hormone signaling inhibitor *via* high-throughput screening of a chemical library. *Sci. Rep.* **10**, 18413 (2020).
24) T. Kayukawa, K. Furuta, K. Yonesu and T. Okabe: Identification of novel juvenile-hormone signaling activators *via* high-throughput screening with a chemical library. *J. Pestic. Sci.*, **46**, 53–59 (2021).
25) L. Bittova, P. Jedlicka, M. Dracinsky, P. Kirubakaran, J. Vondrasek, R. Hanus and M. Jindra: Exquisite ligand stereoselectivity of a *Drosophila* juvenile hormone receptor contrasts with its broad agonist repertoire. *J. Biol. Chem.* **294**, 410–423 (2019).
26) M. Jindra and L. Bittova: The juvenile hormone receptor as a target of juvenoid "insect growth regulators". *Arch. Insect Biochem. Physiol.* **103**, e21615 (2020).
27) T. Yokoi, T. Nabe, S. Horoiwa, K. Hayashi, S. Ito-Harashima, T. Yagi, Y. Nakagawa and H. Miyagawa: Virtual screening identifies a novel piperazine-based insect juvenile hormone agonist. *J. Pestic. Sci.*, **46**, 68–74 (2021).

28) T. Yokoi, T. Nabe, C. Ishizuka, K. Hayashi, S. Ito-Harashima, T. Yagi, Y. Nakagawa and H. Miyagawa: Transcription-inducing activity of natural and synthetic juvenile hormone agonists through the *Drosophila* Methoprene-tolerant protein. *Pest Manag. Sci.* **76**, 2316–2323 (2020).
29) J. W. Truman and L. M. Riddiford: The origins of insect metamorphosis. *Nature* **401**, 447–452 (1999).
30) M. Jindra: Where did the pupa come from? The timing of juvenile hormone signalling supports homology between stages of hemimetabolous and holometabolous insects. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **374**, 20190064 (2019).
31) D. F. Erezyilmaz, L. M. Riddiford and J. W. Truman: Juvenile hormone acts at embryonic molts and induces the nymphal cuticle in the direct-developing cricket. *Dev. Genes Evol.* **214**, 313–323 (2004).
32) S. Naruse, M. Ogino, T. Nakagawa, Y. Yasuno, A. Jouraku, T. Shiotsuki, T. Shinoda, K. Miura and C. Minakuchi: Ovicidal activity of juvenile hormone mimics in the bean bug, *Riptortus pedestris. J. Pestic. Sci.*, **46**, 60–67 (2021).
33) Y. Ando, K. Matsumoto, K. Misaki, G. Mano, S. Shiga, H. Numata, T. Kotaki, T. Shinada and S. G. Goto: Juvenile hormone III skipped bisepoxide, not its stereoisomers, as a juvenile hormone of the bean bug *Riptortus pedestris. Gen. Comp. Endocrinol.* **289**, 113394 (2020).
34) R. Parthasarathy and S. R. Palli: Stage-specific action of juvenile hormone analogs. *J. Pestic. Sci.*, **46**, 16–22 (2021).
35) E. Satoh, R. Kasahara, K. Fukatsu, T. Aoki, H. Harayama and T. Murata: Benzpyrimoxan: design, synthesis, and biological activity of a novel insecticide. *J. Pestic. Sci.*, **46**, 109–114 (2021).