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Juvenile hormone (JH) is a sesquiterpenoid of vital importance for insect development, yet the molecular basis of JH signaling remains obscure, mainly because a bona fide JH receptor has not been identified. Mounting evidence points to the basic helix–loop–helix (bHLH)/Per-Arnt-Sim (PAS) domain protein Methoprene-tolerant (Met) as the best JH receptor candidate. However, details of how Met transduces the hormonal signal are missing. Here, we demonstrate that Met specifically binds JH III and its biologically active mimics, methoprene and pyriproxyfen, through its C-terminal PAS domain. Substitution of individual amino acids, predicted to form a ligand-binding pocket, with residues possessing bulkier side chains reduces JH III binding likely because of steric hindrance. Although a mutation that abolishes JH III binding does not affect a Met–Met complex that forms in the absence of methoprene, it prevents both the ligand-dependent dissociation of the Met–Met dimer and the ligand-dependent interaction of Met with its partner bHLH-PAS protein Taiman. These results show that Met can sense the JH signal through direct, specific binding, thus establishing a unique class of intracellular hormone receptors.

structure modeling | insecticide action | metamorphosis | *Tribolium* | *Drosophila*

Juvenile hormone (JH) prevents adult transition (metamorphosis) of insect larvae until they have attained an appropriate stage (1, 2), and it typically stimulates oogenesis in adult females (3). How JH achieves its function remains unclear, mainly because a JH receptor has long eluded identification (4). The lipophilic nature of the sesquiterpene JH suggests an intracellular receptor, yet none of the known insect nuclear hormone receptors have been linked with the biological function of JH. A screen for *Drosophila* mutants resistant to methoprene (5), a JH mimic and a widely used insecticide (6), uncovered the Methoprene-tolerant (Met) protein containing a basic helix–loop–helix (bHLH) motif followed by two Per-Arnt-Sim (PAS) domains (7). Recombinant *Drosophila* Met was shown to bind JH at physiological (nanomolar) concentrations and to mediate a weak JH- and methoprene-dependent transcriptional activation in vitro (8). However, *Met*-null mutant flies were viable and fertile (5), leaving the notion that Met is a putative JH receptor unsupported with an anticipated developmental phenotype. Latest reports show that, in *Drosophila*, Met might functionally overlap with its paralog, encoded by the *germ cell-expressed* (*gce*) gene. Gce can increase sensitivity of *Met*-null mutants to methoprene (9), and only simultaneous loss of both Met and Gce is lethal (10). However, the actual mode of interaction between JH/methoprene and Met or Gce still remains unclear.

Knockdown of the single *Met* gene in the flour beetle *Tribolium castaneum* induced beetle larvae to pupate before reaching their final instar (11), producing a precocious metamorphosis phenotype similar to that caused by loss of JH itself (12). Conversely, removal of Met precluded inhibition of adult development by exogenous JH (11, 13). The studies in *Tribolium* have thus provided the missing evidence that Met is an essential, JH-dependent repressor of insect metamorphosis. Recently, premature degeneration of the fat body was observed in *Drosophila* larvae that either were deprived of JH or lacked both Met and Gce, and addition of a JH mimic (pyriproxyfen) could remedy only deficiency of JH but not the loss of Met and Gce (10). Nevertheless, Met and Gce may not always act redundantly because precocious metamorphic development occurring within the nervous system of either JH-deficient or *Met*-null (gce^{+}) *Drosophila* prepupae was only suppressed to a minor degree in *Met* mutants treated with pyriproxyfen (14). Met exerts its anti-metamorphic effect at least in part via JH-inducible activation of the *Krüppel homolog 1* (*Kr-h1*) gene (13).

bHLH-PAS proteins typically form heterodimeric transcription factors. The vertebrate aryl hydrocarbon receptor (AhR) requires activation by a ligand bound to its C-terminal PAS domain (PAS-B) to combine with the AhR nuclear translocator (Arnt) and to activate transcription (15). Similarly, Met has been recently shown to form a JH-dependent transcriptionally active complex with another member of the bHLH-PAS family, termed FISC (16) or SRC (17), the latter name reflecting its homology with the mammalian steroid receptor coactivator 1 (SRC-1)/NCoA-1/p160 (18). Following the FlyBase nomenclature, we will refer to this protein as Taiman.

Despite the recent progress, Met has not yet been generally recognized as a bona fide JH receptor. The high-affinity binding of JH by Met (8) has neither been verified nor extended to other species, and a ligand-binding domain of Met has not been characterized. Consequently, it could not be ascertained whether the JH-dependent interaction between Met and Taiman requires the hormone to be bound to a specific ligand-binding site. Here, we show that *Tribolium* Met binds JH and its mimics with high affinity through a well-conserved hydrophobic pocket within its PAS-B domain. We identify specific amino acid residues responsible for JH binding and demonstrate that the ligand-binding capacity is necessary for interaction of Met with its partner Taiman.

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Results and Discussion

Binding of JH III to Met Homologs. The in vitro-translated product of the *Drosophila Met* gene was shown to bind JH III with a K_d of 5.3 nM (8). Our first goal was to reproduce this result and see whether it applied to homologous proteins in *Drosophila melanogaster* and other species. Met from the beetle *Tribolium* was our primary interest because we have defined Met as an essential transducer of the JH signal during metamorphosis in this model (11). In all hormone-binding assays, we have been using proteins with an N-terminal Myc epitope tag, which were translated from DNA optimized for mammalian codon use in rabbit reticulocyte lysates. This system allowed us to (*i*) ensure standard expression of different proteins, (*ii*) track the amount and stability of each protein in every assay, and (*iii*) eliminate endogenous JH binders occurring in insect systems.

In agreement with the previous report (8), we detected low but reproducible binding of [^{3}H]JH III to the *Drosophila* Met (DmMet) protein (Fig. 1*A*). Unexpectedly, the activity of DmMet appeared much weaker compared with its paralog DmGce (Fig. 1*A*), which had not been previously tested. The capacity of both *Drosophila* Met and Gce to bind JH III agrees with the ability of Gce to restore methoprene sensitivity in *Met*-null flies (9) and supports the view that Met and Gce functionally overlap (10). The full-length Met protein from the beetle *Tribolium* showed robust binding of JH III (Fig. 1*A*). Saturation experiments determined the K_d value of 2.94 ± 0.68 nM (Fig. 1*B*). Therefore, *Tribolium* Met binds JH III with an affinity comparable to that reported for *Drosophila* Met (8).

To test whether Met might have the JH-binding capacity in evolutionarily distant insects, we chose the firebrat, *Thermobia domestica* (Zygentoma), which represents a basal wingless insect lineage and is known to possess endogenous JH III (19). We have cloned an ortholog of *Met* from the firebrat (20). The in vitro-translated PAS-B domain of *Thermobia* Met proved to be a potent JH III binder (Fig. 1*A*), suggesting that the capacity of Met to sense the JH signal resides in the conserved C-terminal PAS domain and that it predates the evolution of insect metamorphosis.

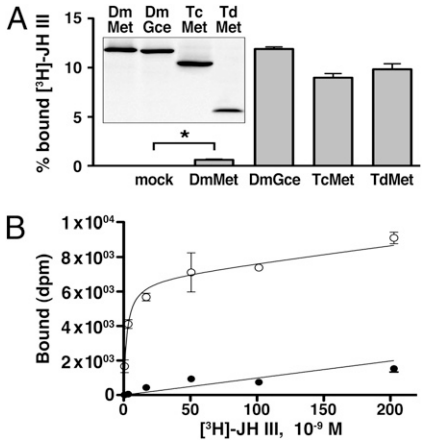

Fig. 1. *Tribolium* Met and its orthologs bind JH III. (*A*) Myc-tagged full-length proteins from *D. melanogaster* (Dm) and *T. castaneum* (Tc) and the PAS-B region from *T. domestica* (Td) were translated in vitro (*Inset*, immunoblot) and incubated with 0.5 pmol of [^{3}H]JH III. Total binding is compared against reticulocyte lysate without DNA (mock). The value obtained for DmMet is significantly higher than mock (Mann–Whitney test, $P < 0.05$; $n = 4$). (*B*) Full-length *Tribolium* Met was incubated with increasing concentrations of [^{3}H]JH III in the absence (○, total binding) or presence (●, nonspecific binding) of a 100-fold molar excess of unlabeled JH III. Each data point is mean ± SD of two to four assays, and the saturation curve shown is average of four independent experiments. The calculated K_d is 2.94 ± 0.68 nM.

PAS-B Domain of *Tribolium* Met Specifically Binds JH III and Its Mimics. To determine which part of the *Tribolium* Met protein is responsible for binding JH III, we systematically deleted the conserved domains (Fig. 2*A* and Fig. S1). Testing the truncated proteins in the ligand-binding assay revealed that the N-terminal half of Met, including the bHLH and PAS-A domains (amino acids 1–240), was not required and had no JH-binding activity alone (Fig. 2*B*). Only constructs containing the PAS-B domain were capable of binding the hormone. The C-terminal region, which shows poor sequence conservation (residues 387–516), was dispensable for hormone binding, but its removal lowered the protein yield (Fig. S1), likely causing lower JH binding in constructs Met(1–386) and Met(240–386) (Fig. 2*B*). Because the C-terminal region improved protein expression, we performed further analyses either on full-length Met(1–516) or on Met (240–516), which includes the PAS-B domain and the native protein end. Taiman, the bHLH-PAS dimerization partner of Met (16, 17), did not bind JH III (Fig. 2*B*). To verify the ligand-binding capacity of Met PAS-B in another type of assay, we subjected Met to equilibrium dialysis in the presence of radiolabeled JH III. The hormone specifically accumulated in the dialysis compartment containing Met(1–516) and Met(240–516) but not Met(1–240), which lacks the PAS-B domain (Fig. S2).

Saturation assays determined that Met(240–516) bound JH III with an average K_d of 12.3 ± 0.62 nM (Fig. 3*A*), an affinity slightly lower than that of the entire protein (Fig. 1*B*). Methoprene and pyriproxyfen, known to be effective juvenoids in *Tribolium* (11, 13, 21), competed against JH III in binding to Met(240–516), whereas the inactive JH precursor farnesol did not (Fig. 3*B*). K_i values of 388 ± 52 nM for methoprene and 4.75 ± 0.86 nM for pyriproxyfen suggested an affinity ranking of pyriproxyfen > JH III > methoprene for Met(240–516). The above data show that the PAS-B domain of Met is necessary and sufficient for specific, high-affinity ligand binding and discriminates between biologically active and inert compounds.

Pyriproxyfen Is a Potent Met Agonist. Unlike methoprene, pyriproxyfen has a chemical structure unrelated to natural JH (6). Because pyriproxyfen is both a potent ligand of Met (Fig. 3*B*) and more effective than methoprene as insecticide against *Tribolium* (21), we tested whether pyriproxyfen exerted a specific biological effect in vivo through Met. Indeed, pyriproxyfen induced expression of a well-characterized Met target gene, *Kr-h1*, in *Tribolium* pupae. This induction required Met because it was abolished in animals lacking the Met protein (Fig. 3*C*). Consistent with its higher affinity to Met(240–516), pyriproxyfen

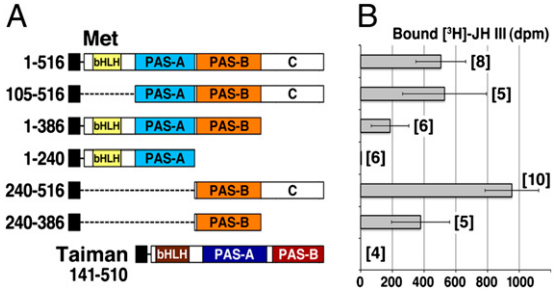

Fig. 2. The PAS-B domain of Met is necessary and sufficient for JH III binding. (*A*) Deletion constructs representing the individual domains of *Tribolium* Met and Taiman proteins tagged with the Myc epitope (black boxes) were translated in vitro (for immunoblot, see Fig. S1). Numbers indicate amino acid positions; 1–516 is the entire Met protein. (*B*) JH III-binding activities are plotted next to the respective proteins as total radioactivity bound. Values are mean ± SD of several independent repeats (*n* numbers are in brackets).

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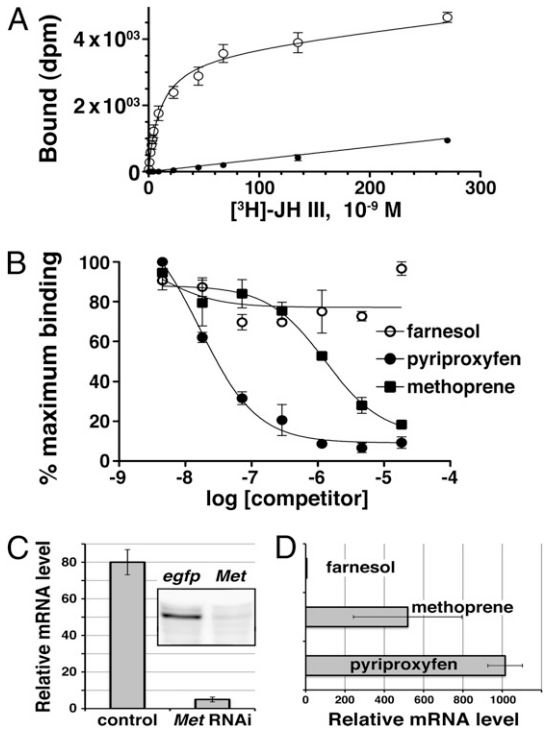

Fig. 3. Met selectively binds JH III and its mimics and mediates the effect of pyriproxyfen in vivo. (*A*) In vitro-translated Met PAS-B was incubated with [^{3}H]JH III in the absence (○, total binding) or presence (●, nonspecific binding) of a 100-fold molar excess of cold JH III. The saturation curve shown is average of six independent experiments. The calculated K_d is 12.3 ± 0.62 nM. (*B*) Met PAS-B was incubated with 2 pmol of [^{3}H]JH III in the presence of increasing concentrations of the indicated compounds. The competition curves shown are average of three independent experiments. The calculated dissociation constants (K_i) are 388 ± 52 nM for methoprene and 4.75 ± 0.86 nM for pyriproxyfen; farnesol did not significantly compete for binding. (*C*) At 3 d after injection with *egfp* (control) or *Met* dsRNA, *Tribolium* pupae were treated with pyriproxyfen and tested for *Kr-h1* mRNA expression 12 h later. *Inset* shows RNAi knockdown of the Met protein in these pupae. Data are mean ± SD from six animals. (*D*) *Kr-h1* mRNA levels were assessed in *Tribolium* pupae at 8 h after treatment with 0.1 mM solutions of the indicated compounds. Data are mean ± SD from n = 4 pupae; the difference between pyriproxyfen and methoprene is significant at P = 0.03 (Student's t test).

appeared to be more potent than methoprene in inducing *Kr-h1* transcription (Fig. 3*D*). Therefore, like JH III or its structural analog methoprene, pyriproxyfen activates JH-dependent gene expression through the same receptor protein. These data establish pyriproxyfen as a Met agonist and Met as a target of chemically diverse insecticides that mimic the effect of JH.

Structural Models Predict a JH-Binding Pocket Within the PAS-B Domain of Met. To understand how the structure of Met might accommodate the hormonal ligand, we modeled the *Tribolium* Met PAS-B domain based on the crystal structure of hypoxia-inducible factor 2α (HIF2α) PAS-B (22) as a homologous template (Fig. S3). The obtained model contained an elongated internal cavity of 625 Å^3 that extended from helix Cα to Fα and presented an opening between the Fα-helix and the β-sheet (Fig. 4*A*). Secondary structure elements of the PAS-B domain contributed to this pocket with mainly hydrophobic residues (Fig. 4*B*). Both the size and the hydrophobic nature of the cavity fit the expected role of binding small hydrophobic ligands.

Computational docking of JH III to the Met PAS-B model led to several solutions, which all filled the bottom part of the pocket near the Cα-helix (Fig. S4*A*). The best docking result corresponded to a theoretical affinity of −7.4 kcal/mol and showed JH III forming a single hydrogen bond with the Tyr-252 side-chain hydroxyl group (N-terminal β-strand Aβ) through its epoxide moiety (Fig. 4*C*). Docking of the chemically disparate but biologically active pyriproxyfen molecule into the Met PAS-B domain produced a single best solution with a theoretical affinity of −9.2 kcal/mol. Pyriproxyfen in its position overlapped with JH III and contacted the side chains of Tyr-252 and Lys-311 through hydrogen bonds involving two of its ether groups (Fig. S4*B*). The good fit of pyriproxyfen to the PAS-B model corresponded with its ability to effectively compete with JH III for binding Met (240–516) (Fig. 3*B*).

Mutations Within the Ligand-Binding Pocket Disrupt JH III Binding. To test the model of JH binding to Met, we changed several of the residues whose side chains point toward the ligand (Fig. 4*C*). These mutations (Fig. S5) were to amino acids with larger side chains but similar physicochemical properties to block ligand binding by steric hindrance. All versions of Met(240–516) harboring the individual mutations were expressed to the same extent and remained stable throughout the JH III-binding assay (Fig. S6). Mutations T254Y, V280F, V297F, T330Y, and C347M resulted in total or nearly total loss of detectable JH III binding; I262F and L318F reduced it to 15% and 30%, respectively, of WT PAS-B activity (Table 1). In contrast, substituting phenylalanine for the conserved valine residues at positions 346 and 348, whose side chains point away from the ligand-binding pocket, only reduced JH III binding to 85% and 91% of the WT, respectively (Table 1). To verify whether mutations disrupting JH III binding of Met PAS-B had the same effect in the context of the entire Met protein, we introduced mutations of two

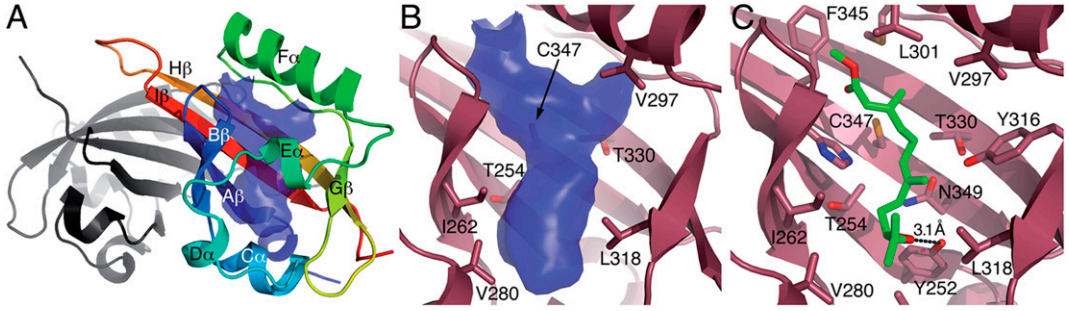

Fig. 4. Model of the ligand-binding cavity of the *Tribolium* Met PAS-B domain. (*A*) Overall structure of Met PAS-B (blue, N terminus; red, C terminus) with the cavity. Position of a hypothetical heterodimeric partner, here represented by Arnt as in the HIF2α–Arnt crystal structure PDB ID 3F1P (22), is shown in gray. (*B*) A closer view of the pocket relative to the amino acid residues mutated in this study. (*C*) Docking model of a Met–JH III complex. A hydrogen bond (dotted line) is predicted between the hydroxyl group of Tyr-252 and the epoxide moiety of JH III. Orientation is the same in all models.

Table 1. Effect of mutations within the PAS-B domain of *Tribolium* Met on JH III binding

Mutation	WT Met(240–516) binding, %	*n*	WT Met(1–516) binding, %	*n*
WT	100 ± 11.8	9	100 ± 3.4	3
Y252F	110.9 ± 15.2	6		
Y252W	21.5 ± 3.1	7		
T254Y	0.4 ± 0.7	3		
I262F	14.9 ± 2.3	7		
V280F	1.0 ± 0.9	3	0.9 ± 0.3	3
V297F	0.7 ± 0.8	9	1.4 ± 1.3	4
L318F	29.7 ± 8.6	7		
T330Y	2.0 ± 1.5	3		
V346F	85.3 ± 7.2	5	90.0 ± 5.8	3
C347M	2.1 ± 2.6	4		
V348F	91.2 ± 5.3	6		
Mock	0.6 ± 1.1	6		

selected amino acids residing in opposite corners of the ligand-binding cavity, V280F and V297F (Fig. 4*B*), into Met(1–516). Both mutations (but not V346F) abolished JH III binding (Table 1) by the full-length Met protein. The mutant and WT Met(1–516) proteins showed equal expression levels and stability (Fig. S6).

Interestingly, some of the amino acids critical for JH binding in Met occur in positions corresponding to residues within the ligand-binding pocket of AhR PAS-B, which are important for binding of the dioxin (TCDD) ligand (23, 24). Mutations of T283, homologous to T254 in Met (Fig. S7), abolish TCDD binding. AhR mutations P291F and C327A, which affect residues at positions occupied by I262 and V297, respectively, in *Tribolium* Met (Fig. 4*C*), strongly reduce TCDD binding. Finally, substitution of A375 in the center of the AhR ligand-binding pocket with bulkier residues prevents TCDD binding because of steric hindrance (23). A375 corresponds to C347, which is invariant in Met proteins (Figs. S5 and S7).

Although docking solutions for both JH III and pyriproxyfen involved a hydrogen bond with Tyr-252, contribution of this H bond was not critical because mutation Y252F did not reduce JH binding (Table 1). However, tryptophan occurs in place of Tyr-252 in the true bugs, *Pyrrhocoris apterus* (20) and *Rhodnius prolixus* (Hemiptera) (Fig. S5), and the Y252W substitution lowered JH III binding to 21.5% (Table 1). Interestingly, a closely related bug species, *Plautia stali*, possesses a skipped bisepoxide type of JH (25), suggesting that the tryptophan residue might reflect the structural difference of the bug JH.

Ligand-Dependent Protein–Protein Interactions. Met proteins from *Drosophila* and the *Aedes* mosquito form homodimers (and a heterodimer with Gce in *Drosophila*) in the absence of JH III or methoprene; addition of either compound leads to dissociation of the complexes (16, 26). Using immunoprecipitation in transfected human cells, we found that *Tribolium* Met also formed a dimer that dissociated upon methoprene addition (Fig. 5*A*). However, this methoprene-induced dissociation was partially prevented if one of the Met monomers carried the V297F mutation that abolished JH binding, and, when both monomers were mutated, the complex became resistant to methoprene (Fig. 5*A*). Immunoprecipitation of Met(240–516) proteins showed that the PAS-B domain alone was sufficient for dimer formation and that the JH-dependent inhibition of Met dimerization resided within the PAS-B domain itself (Fig. 5*B*). Although Met

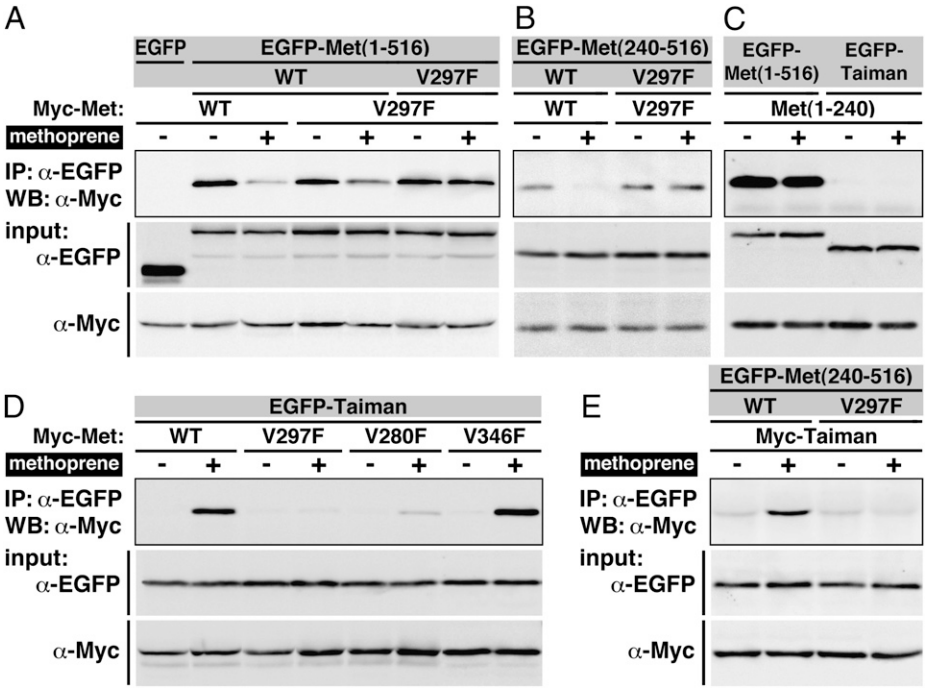

Fig. 5. Ligand-dependent protein interactions of *Tribolium* Met. Proteins N-terminally tagged with either EGFP (shaded) or the Myc epitope were coexpressed in human HEK293 cells. Methoprene (1 μM) or ethanol was added to cell cultures at 1 h before lysis. Cell lysates were subjected to immunoprecipitation (IP, outlined) with an anti-EGFP serum, and interacting proteins were detected on Western blots (WB) with an anti-Myc antibody. Input panels represent 10% of the initial material. Methoprene disrupted homophilic complexes of full-length Met(1–516) (*A*) or PAS-B Met(240–516) (*B*) proteins but not of their V297F mutant versions lacking the JH binding capability. Binding of Met(1–240) bHLH and PAS-A domains to full-length Met was insensitive to methoprene (*C*). Interaction of full-length Met (*D*) or its PAS-B domain (*E*) with Taiman required methoprene and was prevented by mutations that abolish binding of JH.

(1–240) harboring the bHLH and PAS-A regions also dimerized, it did so in a methoprene-insensitive manner (Fig. 5*C*).

The fact that the single point mutation V297F renders the Met–Met complex resistant to the effect of methoprene shows that the dissociation is induced by methoprene through the specific ligand-binding pocket within PAS-B. This process might involve a ligand-induced conformational change of Met that is incompatible with formation of a homophilic complex. The ability of Met V297F to dimerize in the absence of methoprene also confirms that the failure of this mutant to bind JH III is not attributable to a compromised integrity of the protein.

bHLH-PAS proteins typically act as heterodimers (15), and Met proteins from *Aedes* and *Tribolium* have been recently shown to activate gene expression upon JH-dependent physical interaction with Taiman (16, 17). These results have suggested that the Met–Taiman dimer might be an active JH receptor, but the mechanism through which JH induces Met to bind Taiman remains unclear. We addressed this question by using Met mutants incapable of binding JH. The WT *Tribolium* Met bound Taiman only in the presence of methoprene (Fig. 5*D*), confirming the published data (16, 17). By contrast, two mutations that abolished JH III binding, V297F and V280F (Table 1 and Fig. 4*C*), severely reduced the capacity of Met to respond to methoprene by binding Taiman, whereas the neutral V346F substitution left the interaction unaffected (Fig. 5*D*). The same effect of V297F was observed with the truncated PAS-B domain (Fig. 5*E*). These data demonstrate that the interaction between Met and Taiman specifically depends on methoprene binding to the PAS-B domain of Met. Therefore, the critical role of PAS-B in regulating heterodimer formation is common to insect Met and mammalian bHLH-PAS proteins such as HIF2α and Arnt (27).

Together with other recent advances (16, 17, 26), our results support a model in which unliganded Met occurs as a presumably inactive homodimer. Upon JH binding to the PAS-B domain, Met undergoes a conformational change that (*i*) liberates Met from the homophilic complex and (*ii*) allows it to bind Taiman. By sensing JH and forming a ligand-dependent complex with a partner of its own bHLH-PAS family, Met establishes a unique class of intracellular hormone receptors. Its action resembles type II nuclear receptors, whereby a ligand-specific sensor (such as a thyroid hormone or retinoic acid receptor) combines with a versatile heterodimer partner, the retinoid X receptor RXR (28). Although the exact mechanism of JH-dependent activation of Met has yet to be determined, the present study sheds light on the long-elusive problem of JH reception, including the action of insecticidal JH mimics.

Methods

DNA Constructs and Protein Expression. DNA sequences encoding all Met and Gce proteins or their truncated and mutant versions were synthesized for optimal mammalian codon use (GenScript); *taiman* cDNA was cloned from *Tribolium* larval RNA by RT-PCR. Appropriate DNA regions were cloned into the pK-Myc-C2 vector containing a T7 promoter and an N-terminal Myc epitope tag (29). Proteins were produced with the TnT Quick Coupled in vitro transcription/translation system (Promega) with 400 ng of template plasmid per 50-μL reaction. The efficiency of translation was assessed on immunoblots with the mouse anti-Myc antibody 9E10 (Roche).

Ligand-Binding Assays. Racemic (*RS*)-tritiated JH III (10–20 Ci·mMol^{-1}) was from Perkin-Elmer and racemic JH III, pyriproxyfen, *trans,trans*-farnesol, and methoprene were from Sigma-Aldrich. Dextran-coated charcoal (DCC) assays were performed as described (8, 30), and integrity of radiolabeled JH III throughout the assay was verified (*SI Methods* and Fig. S8). Nonspecific binding was determined in DCC assays with a 100-fold molar excess of unlabeled ligand in addition to [^{3}H]JH III. Dissociation constants (K_d) were determined by nonlinear regression from total and nonspecific binding data of saturation experiments by using GraphPad Prism 5.00 (GraphPad Software) on the assumption that JH III binds to a single site and that both the 10*R*- and 10*S*-JH III isomers bind equally. For competition assays, the K_i of JH mimics was calculated by using the "one site fit K_i" model.

Immunoprecipitation. *Tribolium* Met and Taiman proteins were expressed with N-terminal EGFP or Myc epitope tags from pEGFP-C2 (Clontech) or pK-Myc-C2 vectors, respectively, by transient transfection in the HEK293 cells. At 1 h before cell harvesting, cells were treated with 1 μM methoprene (or ethanol for control) and then lysed (*SI Methods*). The lysate was applied (with or without methoprene) to Dynabeads Protein G (Invitrogen) pre-bound with rabbit anti-EGFP antiserum. Input and bound proteins were detected with mouse anti-GFP (Sigma-Aldrich) and anti-Myc 9E10 (Roche) antibodies on immunoblots.

Protein Structure Modeling and Ligand Docking. The *Tribolium* Met PAS-B domain (Leu-240–Leu-358) structure was modeled with Modeler version 9.9 software (31) with HIF2α PAS-B crystal structure (PDB ID 3F1P) (22) as a homologous template. Among 10 models generated, the one with the lowest objective function was retained for ligand docking with AutoDock Vina (32) after the receptor and ligand files were prepared with AutoDock Tools (33). The volume of the cavity of the modeled PAS-B was calculated by using CASTp with default parameters (34). Figures were prepared with Pymol version 0.99 (DeLano Scientific).

RNAi, Hormonal Treatments, and mRNA Expression Analysis. Within 12 h after ecdysis, *Tribolium* pupae were injected with *Met* or control (*egfp*) dsRNA and after 3 d were treated with acetone-diluted 0.1 mM farnesol, methoprene, or pyriproxyfen as described (11). Total RNA was isolated from individual pupae and subjected to quantitative RT-PCR with *Kr-h1* primers (13) by using the iQ SYBR Green Supermix kit and the C1000 Thermal Cycler (Bio-Rad). Data were normalized to the relative levels of ribosomal protein (Rp49) mRNA.

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