Supporting Information

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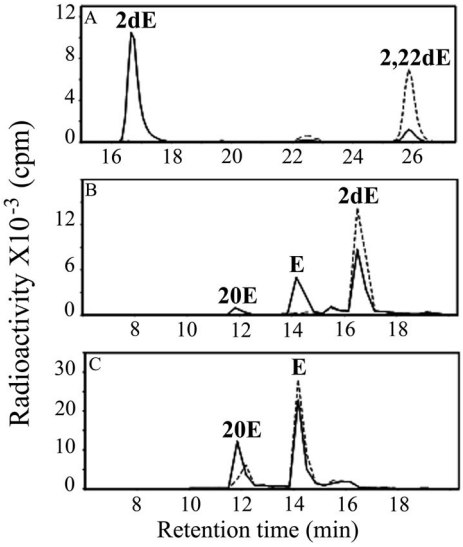

Fig. S1. Catalytic activities of AgCYP302A1, AgCYP315A1, and AgCYP314A1. Cell transfection and incubation with radiolabeled substrates and chromatographic analyses. Schneider *Drosophila* SL2 cell line was maintained at 22 °C in Schneider medium supplemented with 10% heat-inactivated FCS and antibiotics (100 μg/ml streptomycin and 60 μg/ml penicillin). Dimethyl dioctadecyl ammonium bromide (96 μg/plate) was used for transient transfection in 75 cm^2 culture plates. Cells (2.2×10^7/well) were transfected with 5.5 μg pCaSpeR-actin-Ag*CYP302A1*, pIB/V5-Ag*CYP315A1*, pIB/V5-Ag*CYP314A1*, or pCaSpeR-actin-GFP (negative control for metabolism experiments) in 11 ml medium with serum and antibiotics. Transfected cells were first incubated for 3 days at 22 °C to allow protein expression and further incubated for 2 days with 0.2 μCi radiolabeled [22,23 $^3H_2$]2,22dE (specific activity of $\approx$20 Ci/mmol) for testing CYP302A1 activity, with 0.1 μCi [23,24 $^3H_4$]2dE (specific activity of $\approx$25 Ci/mmol) for CYP315A1 and with 0.2 μCi [23,24 $^3H_4$]E (specific activity of 90 Ci/mmol) for CYP314A1. [^{3}H]2,22dE and [^{3}H]2dE were gifts from C. Hétru and J. Hoffmann (Strasbourg, France), and tritiated E was purchased from Perkin-Elmer. After incubation, media plus cells were stored at $-$20 °C. To analyze the conversion by transfected cells, radiolabeled ecdysteroids were extracted from incubation media and cells by C_{18}-Sep Pak cartridges and eluted in methanol. Reference unlabeled ecdysteroids (1 μg) were added and samples were dried. After re-suspension in methanol:water:TFA (500:499:1, vol/vol/v), samples were further analyzed by RP-HPLC using a C_{18} column (Spherisorb 5ODS2, 250 $\times$ 4.6 mm) and eluted using a step-gradient of solvent B (acetonitrile:isopropanol, 5:2, vol/vol) in A (0.1% TFA in water): 0 to 2 min from 15% to 25%, 2 to 8 min at 25%, and 8 to 28 min from 25% to 75% for experiments with [^{3}H]2,22dE; 0 to 6 min at 18% and 6 to 28 min from 18% to 100% for experiments with [^{3}H]2dE or [^{3}H]E. Conversion of radiolabeled substrates by transfected cells analyzed by RP-HPLC is depicted. (*A*) *CYP302A1*-transfected cells hydroxylated 2,22-dideoxyecdysone (2,22dE) almost exclusively into 2-deoxyecdysone (2dE; solid line, 76.6% of total radioactivity), whereas only a background activity was observed in control cells (dotted line, 2% conversion). (*B*) Cells transfected with *CYP315A1* converted 2dE mainly into E (30.1% of total radioactivity) whereas control cells did not significantly convert 2dE into E (3.3%) or 20E (0.6%). A limited further conversion into 20E (5.2% of total radioactivity) was also observed in *CYP315A1*-transfected cells, which likely corresponds to the endogenous capacity of the SL2 cells to metabolize E into 20E (dotted line, *C*). (*C*) *CYP314A1*-transfected cells metabolized E into 20E (29% of total radioactivity) at a higher rate than the control SL2 cells (15.2% of total radioactivity). Solid line, cells transfected with a candidate gene; dotted line, control cells transfected with GFP. Only relevant fractions are presented.

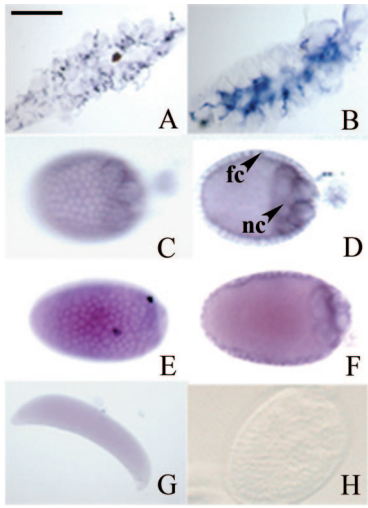

Fig. S2. *In situ* expression pattern of *CYP302A1* in the ovary during the first gonotrophic cycle. (*A*) Ovary from a non-blood-fed female; the ovary is composed of multiple synchronous ovarioles. (Scale bar, 250 μm.) (*B*) Ovary at 6 h PBM. (Scale bar, 250 μm.) (*C* and *D*) Ovariole at 18 h PBM. (Scale bar, 60 μm.) (*E* and *F*) Ovariole at 24 h PBM. (Scale bar, 60 μm.) (*G*) Ovariole at 48 h PBM. (Scale bar, 80 μm.) (*H*) Ovariole at 24 h PBM hybridized with a control sense probe. In ovaries, *CYP302A1* was not detected in the ovarian follicles before the blood meal (*A*) and at 6 h PBM (*B*), but a slight labeling was observed in the germarium, and this may explain the signal observed with RT-PCR. At 18 and 24 h PBM, *CYP302A1* was strongly detected in follicular (*C* and *E*) and nurse (*D* and *F*) cells of primary follicles. At 48 h PBM (*G*), only a weak signal was observed in the vitellogenic follicles. The sense probe was used as a negative control and gave no signal, as shown for a 24 h PBM ovary (*H*). See main article text for *Materials and Methods*.

Table S1. Specific primers used for cloning and RT-PCR experiments

Primers for gene cloning	
*CYP306A1*up1 5′-TTCAAGTAAAGTACAAGGAGAG-3′	*CYP306A1*do1 5′-CGTTTTTAACAGTTTTATTTATTA-3′
*CYP302A1*up1 5′-CGCTCTTACTTTTCTTTCAATA-3′	*CYP302A1*do1 5′-TTATGAAGTTCTCGCCTTC-3′;
*CYP315A1*up1 5′- CGAAGCGGAGTGCGTTGTT-3′	*CYP315A1*do1 5′-CTAGTCCTGCATTTGTGCCA-3′;
*CYP314A1*up1 5′-TGACCACCCAGTATCACTCT-3′	*CYP314A1*do1 5′-GCATCACCCGCTTCCTAATA-3′
Primers for RT-PCR experiments	
*CYP306A1*up2 5′-GTTACTGGAGCGGATTTATT-3′	*CYP306A1*do2 5′-GCTCTTCGCCCAAACACATA-3′
*CYP302A1*up2 5′-CATCCTGCCCACCGACCA-3′	*CYP302A1*do2 5′-CCAAACGGAAGCACCAGGT-3′
*CYP315A1*up1 (see above)	*CYP315A1*do2 5′-CGTCTGTGCGTTTGCTATTC-3′
*CYP314A1*up2 5′-GTTCGGTGTGACCAATCTGA-3′	*CYP314A1*do1 (see above)
*L17*up 5′-TTCCGCATCTCGCTTGGTCT-3′	*L17*do 5′-CCTTCATTTCGCCCTTGTTG-3′