August 2006

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Published in *Insect Biochemistry and Molecular Biology* 36:8 (August 2006), pp. 674–682. doi:10.1016/j.ibmb.2006.05.009

Submitted March 28, 2006; revised May 19, 2006; accepted May 22, 2006; published online May 25, 2006.

Xenobiotic response in *Drosophila melanogaster*: Sex dependence of P450 and GST gene induction

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Abstract: The effect of xenobiotics (phenobarbital and atrazine) on the expression of *Drosophila melanogaster* CYP genes encoding cytochromes P450, a gene family generally associated with detoxification, was analyzed by DNA microarray hybridization and verified by real-time RT-PCR in adults of both sexes. Only a small subset of the 86 CYP genes was significantly induced by the xenobiotics. Eleven CYP genes and three glutathione *S*-transferases (GST) genes were significantly induced by phenobarbital, seven CYP and one GST gene were induced by atrazine. *Cyp6d5*, *Cyp6w1*, *Cyp12d1* and the ecdysone-inducible *Cyp6a2* were induced by both chemicals. The constitutive expression of several of the inducible genes (*Cyp6a2*, *Cyp6a8*, *Cyp6d5*, *Cyp12d1*) was higher in males than in females, and the induced level similar in both sexes. Thus, the level of induction was consistently higher in females than in males. The female-specific and hormonally regulated yolk protein genes were significantly induced by phenobarbital in males and repressed by atrazine in females. Our results suggest that the numerous CYP genes of *Drosophila* respond selectively to xenobiotics, providing the fly with an adaptive response to chemically adverse environments. The xenobiotic inducibility of some CYP genes previously associated with insecticide resistance in laboratory-selected strains (*Cyp6a2*, *Cyp6a8*, *Cyp12d1*) suggests that deregulation of P450 gene expression may be a facile way to achieve resistance. Our study also suggests that xenobiotic-induced changes in P450 levels can affect insect fitness by interfering with hormonally regulated networks.

Keywords: Phenobarbital, Atrazine, DNA microarray, CYP gene, Glutathione *S*-transferase, Esterase, Induction, Resistance

1. Introduction

Multigene families encoding cytochromes P450, glutathione *S*-transferases and esterases are thought to provide animals means to fend off xenobiotic challenges from the environment, such as toxic plant and microbial chemicals encountered in their food or drugs, pesticides and organic pollutants. The multiplicity of P450 enzymes was highlighted 40 years ago by the response of vertebrate tissues to treatment by chemicals such as phenobarbital and 3-methylcholanthrene (Conney, 1967). Biochemically distinct P450 enzymes were revealed by these prototypical inducers. Induction helped characterize most drug-metabolizing enzymes in vertebrates, before their total complement was revealed by genome sequencing. In contrast, the large number of P450 genes in insects and their poor representation in the initial EST collections (Tijet et al., 2001) begged the question of their function. Few genes encoding insect P450s, glutathione *S*-transferases (GST) or esterases have been specifically and directly linked to a role in detoxification or insecticide resistance despite the overwhelming biochemical evidence of their role in these adaptations (Feyereisen, 2005; Ranson and Hemingway, 2005; Oakeshott et al., 2005). A more global approach is therefore needed to characterize these genes. DNA microarray hybridization

is the method of choice to study genome-wide responses, as shown in *Caenorhabditis elegans* (Reichert and Menzel, 2005). In *Drosophila* this technique has been used to identify responses to chemical stressors such as paraquat, H_2O_2 and tunicamycin (Zou et al., 2000; Girardot et al., 2004). Similarly, DNA microarrays have been used to detect genes that are constitutively overexpressed in insecticide-resistant strains from *Drosophila* and mosquitoes obtained from the field or selected further in the laboratory (Daborn et al., 2002; David et al., 2005; Le Goff et al., 2003; Pedra et al., 2004; Vontas et al., 2005). The design of microarrays that selectively represent all members of the detoxification gene families and appropriate controls genes can provide a useful tool to identify inducible genes. Gerhold et al. (2001) have used such a "detox chip" with 300 rat genes represented to study the effects of four known inducers, phenobarbital, 3-methylcholanthrene, dexamethasone and clofibrate on rat liver gene expression. This study described the inducer-specific pattern of transcriptional activation and confirmed the induction of *CYP2B1*, *CYP2B2*, *CYP2C6* as well as *CYP3A12* and *3A2* by phenobarbital.

Induction of drug-metabolizing enzymes, and of cytochrome P450-mediated pesticide metabolism in particular, has been extensively studied in insects (Yu, 1986). Induction of specific genes is now well documented (e.g., *CYP6A1*; Cariño et al., 1992) and induction of P450 genes by a wide variety of chemicals is now amply demonstrated (review in Feyereisen, 2005), although the pattern of response within the whole multigene families or the selectivity of the inducer has yet to be documented.

Here we use a *Drosophila melanogaster* DNA microarray of 319 genes covering in a redundant manner all CYP genes, GST genes and esterase genes, to determine which genes are affected in both males and females by treatment of the flies with phenobarbital and atrazine, a prototypical inducer and a widely used herbicide known to contaminate both terrestrial and aquatic habitats. Our results show that induction is sex- and inducer-specific and that xenobiotic exposure can interfere with hormonally regulated physiological pathways.

2. Materials and methods

2.1. Drosophila stock and induction regime

All fly stocks were maintained at 21 °C on standard corn medium for a 12:12 h dark/light period. Oregon R flies were used as wild-type strain. Emerging flies were separated by sex and maintained on standard diet for 72 h before induction. The flies were then placed on medium containing the inducer at 10 mM for phenobarbital or 5 mM atrazine for 72 additional hours. These concentrations were sublethal during the course of the experiment. After exposure, flies were frozen in liquid nitrogen for subsequent RNA extraction.

2.2. RNA extraction

Total RNA was extracted from 50–100 flies using Trizol Reagent (Invitrogen Life technologies). Three independent extractions were performed on three independent biological replicates.

2.3. Microarray construction

A cDNA microarray was constructed using expressed sequence tags (ESTs) of 1.8 kb average length and gene-specific tags of 0.4 kb average length obtained by PCR amplification and plasmid cloning. This "*Drosophila* toxicology microarray" contained 319 genes that represent all cytochromes P450, glutathione *S*-transferases, esterases, other genes of interest including attacin or Ftz-F1 and several housekeeping genes as controls. Each insert was amplified by PCR with flanking universal primers, purified and then spotted using the ChipWriterPro Virtek (BioRad) spotter at three independent locations on Corning UltraGAPS slides (Corning). All information concerning the array is available at GEO (www.ncbi.nlm.nih.gov, GEO accession GPL3874).

2.4. cDNA preparation and microarray hybridization

cDNA were synthesized from 10 μg of total RNA and labelled with the dyes Cy3- dCTP and Cy5- dCTP (Amersham) using the Pronto! Universal Microarray Hybridization Kit according to the manufacturer's instructions. Each RNA was labelled with Cy3 and Cy5 to perform reverse-labelled replicate arrays (dye swap) because of bias in dye incorporation. One comparison consists of a total of six arrays corresponding to two dye-swap arrays for each of our three biological replicates. We realized a balanced-block design of 24 arrays to compare phenobarbital and atrazine treatments in which female and male were separately treated. Hybridizations were performed at 42 °C for 14 h, followed by post-hybridization washes. Slides were scanned on a Genepix 4000b scanner and signal quantification was performed using the Genepix pro 4.1 software (Axon Instruments).

2.5. Microarray data analysis

Data files from Genepix were converted using Express Converter software and normalized in Microarray Data Analysis System (MIDAS), using two publicly available programs from TIGR (http://www.tigr.org/software). Three steps of normalization were performed in MIDAS, (a) a low-intensity filter in which signal intensity <1000 was removed; (b) a global lowess normalization was applied; (c) a flip dye consistency check, the Cy3 and Cy5 labels were swapped between controls versus treatments to account for potential differences in labelling efficiency. For statistical analysis, each array intensity was log-transformed, centred by their average value and reduced by their standard deviation. The resulting data for each

gene were analyzed with a type III two-way (i.e., sex × treatment) analysis of variance (ANOVA) taking into account the presence of missing values. A gene was considered as differentially expressed if 50% or more of its variance was explained by the treatment effect with a corresponding *P*-value lower than 0.035. Data were removed when there was no consistency for a same gene represented on the array by PCR products of different size, as this may have reflected a different degree of specificity.

2.6. Quantitative real-time PCR

Total RNA (1 μg) was reverse-transcribed using the iScript cDNA Synthesis Kit (BioRad). QPCR reactions were carried out on an Opticon monitor 2 (BioRad) using the Absolute QPCR SYBR green Mix (ABgene). The PCR conditions were as follows: 95 °C for 15 min to activate the hot-start DNA polymerase, followed by 40 cycles of 95 °C for 30 s, 65 °C for 30 s and 72 °C for 30 s. Each reaction was performed in triplicate and the mean of three independent biological replicates was calculated. All results were normalized to the RpL17 mRNA level and calculated using the ΔCt method.

3. Results

Adult flies of both sexes were exposed to phenobarbital and atrazine in their food, and changes in the transcript abundance for P450, GST and esterase genes assayed using microarray hybridization. Table 1, Table 2, Table 3 and Table 4 list the genes that were significantly up- or down-regulated and the ratio of treated versus control transcript levels expressed as fold change for each treatment and for males and females.

Phenobarbital induced seven P450 genes and one GST gene in males. In females, phenobarbital induced ten P450 and two GST genes and it down-regulated two P450 genes and the α-Esterase 3 gene. Six P450 genes as well as GST D2 were induced by phenobarbital in both sexes. Atrazine induced four P450 genes, a GST gene and an esterase gene in males; it repressed one P450 gene, a GST and an esterase gene. In females, atrazine induced six P450 genes. Three P450 genes were induced by atrazine in both sexes.

Only three P450 genes, *Cyp6a2*, *Cyp6w1*, *Cyp12d1*, were induced by both chemicals and in both sexes. The other genes had sex- and/or chemical-selective responses. Surprisingly, the three yolk protein (*Yp*) genes that were part of the set of control genes on the array were found to be strongly induced by phenobarbital in males and were upregulated from virtually undetectable levels in untreated flies. Atrazine down-regulated these same genes in females (Table 4). Further, the Ftz-f1 nuclear receptor gene was significantly down-regulated in females by atrazine. Cytochrome b_5, a redox partner in some microsomal P450 reactions, was induced in males and repressed in females by phenobarbital.

Microarray results were confirmed by qRT-PCR for *Cyp6a2*, *Cyp6d5*, *Cyp6g1*, *Cyp6w1*, *Cyp12d1* and also for *Cyp6a8* that was not included in the microarray experiments for technical reasons. The results (Fig. 1 and Fig. 2) highlight the significant sex-dependent differences in induction with low basal levels in females and more spectacular induction than in males. The strong induction of yolk protein genes was confirmed by qRT-PCR (Fig. 3).

Table 1.
Genes differentially expressed in females flies after phenobarbital treatment (ANOVA)

Category	CG#	Name	% Variance	Log*p* value	Fold change	S.E.
Cytochromes P450	CG9438	Cyp6a2	96.8	−13.3	8.69	0.12
	CG10241	Cyp6a17	96.6	−3.9	7.38	1.01
	CG8345	Cyp6w1	95.0	−11.3	8.25	0.28
	CG3050	Cyp6d5	94.3	−14.5	7.96	0.38
	CG10242	Cyp6a23	90.0	−10.7	4.11	0.21
	CG33503	Cyp12d1	85.8	−4.9	9.75	1.00
	CG8453	Cyp6g1	74.6	−6.0	2.04	0.14
	CG4486	Cyp9b2	74.2	−4.5	2.53	0.23
	CG2060	Cyp4e2	74.1	−6.1	2.38	0.13
	CG3540	Cyp4d4	64.1	−1.8	2.48	0.81
	CG17453	Cyp317a1	62.5	−5.7	0.60	0.09
	CG6730	Cyp4d21	56.7	−4.6	0.52	0.02
GST and esterases	CG4181	GstD2	86.7	−8.9	2.37	0.13
	CG1257	a-Est3	74.8	−2.4	0.49	0.07
	CG4371	GstD7	70.7	−3.9	3.86	0.87
	CG5224	GST	53.2	−5.7	0.65	0.04
	CG6018	Carboxylest. β microsomal	56.4	−1.8	0.64	0.05
Other	CG2140	Cytb5	72.4	−1.9	0.51	0.08
	CG2512	α Tubulin at 84D	63.0	−7.8	0.53	0.04
	CG2099	RpL35A	50.8	−3.3	0.72	0.05

Table 2.
Genes differentially expressed in male flies after phenobarbital treatment (ANOVA)

Category	CG#	Name	% Variance	Log p value	Fold change	S.E.
Cytochromes P450	CG10241	Cyp6a17	83.8	−5.7	2.10	0.40
	CG8345	Cyp6w1	83..1	−8.1	2.04	0.22
	CG10242	Cyp6a23	78.2	−4.7	1.71	0.05
	CG3050	Cyp6d5	76.8	−5.0	1.64	0.06
	CG6816	Cyp18a1	72.4	−6.5	2..21	0.31
	CG33503	Cyp12d1	59.2	−4.6	1.72	0.19
	CG9438	Cyp6a2	49.9	−3.1	1.54	0.01
GST and esterases	CG4181	GstD2	68.2	−5.4	1.35	0.06
Other	CG2979	Yolk protein 2	98.3	−1.2	9.88	
	CG11129	Yolk protein 3	97.3	−4.5	7.65	0.59
	CG2985	Yolk protein 1	90.9	−6.1	11.97	0.92
	CG2140	Cytb5	56.4	−2.1	1.90	0.34
		Tubulin. EST AA949515	61.2	−4.8	0.67	0.07

Table 3.
Genes differentially expressed in male flies after atrazine treatment (ANOVA)

Category	CG#	Name	% Variance	Log p value	Fold change	S.E.
Cytochromes P450	CG4105	Cyp4e3	96.0	−6.0	4.48	0.54
	CG33503	Cyp12d1	91.1	−10.7	4.56	0.11
	CG8345	Cyp6w1	84.5	−6.5	2.53	0.41
	CG9438	Cyp6a2	67.9	−9.7	3.52	0.28
	CG6042	Cyp12a4	60.9	−4.6	0.62	0.05
GST and esterases	CG6917	Est6	83.7	−8.6	0.37	0.03
	CG10339	Esterase	80.7	−1.6	1.39	
	CG18548	GstD10	69.0	−3.8	2.02	0.44
	CG1681	GST	54.9	−3.0	0.75	0.04
Other	CG10146	Attacin A	90.1	−2.3	7.05	3.15
		Tubulin, EST AA949515	83.0	−7.5	0.44	0.02
	CG10602	"Alanyl aminopeptidase"	64.4	−3.5	0.65	0.03

Table 4.
Genes differentially expressed in female flies after atrazine treatment (ANOVA)

Category	CG#	Name	% Variance	Log p value	Fold change	S.E.
Cytochromes P450	CG8345	Cyp6w1	66.0	−3.6	3.12	1.08
	CG9438	Cyp6a2	65.3	−3.4	2.47	0.91
	CG7241	Cyp304a1	60.2	−1.8	1.83	0.02
	CG3050	Cyp6d5	57.1	−3.8	1.53	0.30
	CG33503	Cyp12d1	56.5	−3.0	2.56	0.80
	CG8453	Cyp6g1	49.4	−2.4	1.56	0.25
Other						
	CG10146	Attacin A	70.5	−1.7	0.44	0.11
	CG2985	Yolk protein 1	64.9	−3.6	0.58	0.16
	CG2979	Yolk protein 2	64.1	−3.3	0.38	0.19
	CG2512	αTubulin at 84D	56.8	−3.5	0.66	0.22
	CG11129	Yolk protein 3	53.9	−3.2	0.54	0.03
	CG4059	ftz transcription factor 1	53.3	−2.7	0.53	0.02

4. Discussion

Our results show that the *Drosophila* genes coding for the "drug-metabolizing enzymes" (DME) are not uniformly responsive to xenobiotics. Only five P450 genes, and one GST gene, were induced by the two chemicals used in this study, whereas another handful of genes showed a more selective pattern of transcriptional variation. Even though our ar

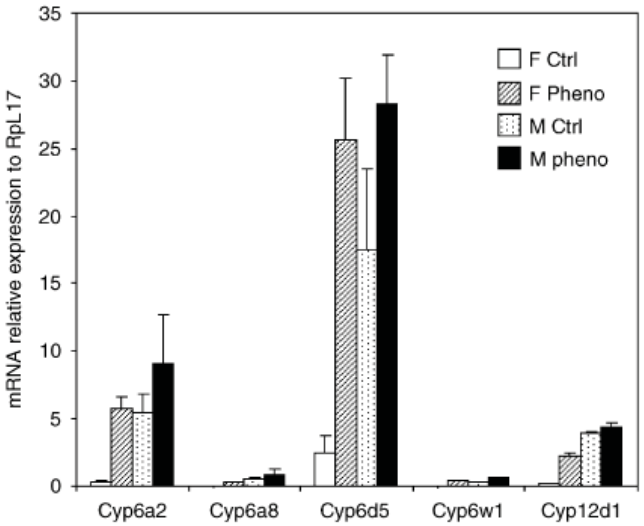

Figure 1. Effects of phenobarbital on CYP mRNA levels in male and female *Drosophila* . Validations of microarray data by qPCR. Data presented are the ratio of Cyp to Rpl17.

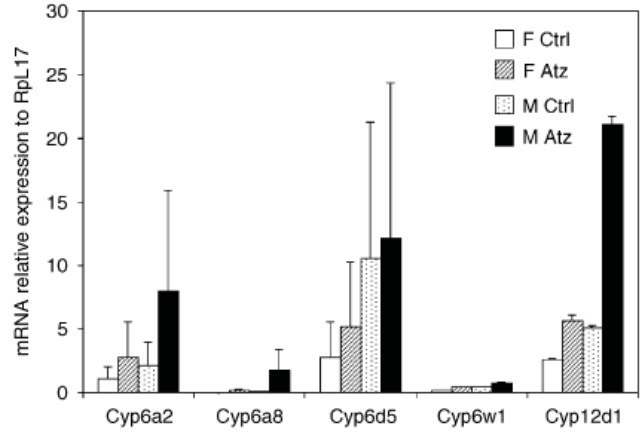

Figure 2. Effects of atrazine on CYP mRNA expression in male and female *Drosophila* . Validations of microarray data by qPCR. Data presented are the ratio of Cyp to Rpl17.

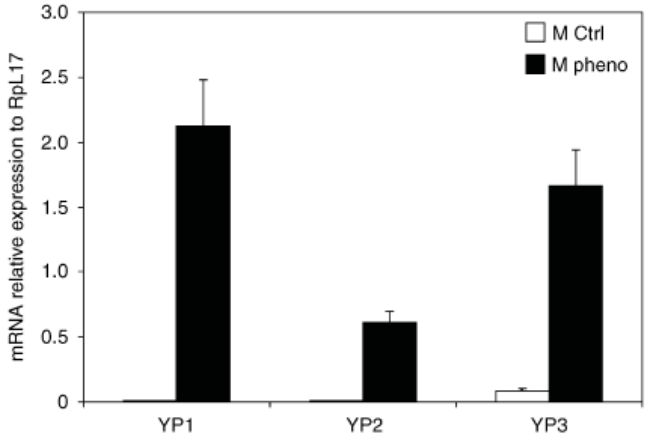

Figure 3. Effects of phenobarbital on yolk protein mRNA levels in adult males of *Drosophila* . Validations of microarray data by qPCR. Data presented are the ratio of YP to Rpl17.

rays cover by design only a small proportion of all *Drosophila* genes, the results clearly identify a subset of genes that respond to xenobiotics and support the usefulness of such thematic microarrays. Our study reveals that some of the genes regulated by xenobiotics have been associated with insecticide resistance or shown to be involved in resistance. Further we show that there is a strong sex bias in the induction, and that treatment by xenobiotics also disrupts the normal expression of genes known to be involved in endocrine processes.

The selective detection of transcriptional differences of closely related members of large gene families can be a difficult task, that was addressed for P450s in our study by the use of multiple DNA features of different sizes for each gene on the array, as well as by additional techniques, such as qRT-PCR. Affymetrix chips can distinguish genes up to a threshold of approximately 90% DNA identity (Gerhold et al., 2001), whereas cDNA/PCR-based arrays can distinguish P450 genes up to 80% DNA identity (Xu et al., 2001). We therefore used qRT-PCR normalized against Rpl17 expression to confirm the results of the microarrays. The results obtained are consistent with each other, although qRT-PCR is, as expected, a more sensitive tool. The use of a microarray dedicated to a subset of genes has already proven useful in detecting transcriptional changes that are associated with insecticide resistance in *Drosophila* (Daborn et al., 2002; Le Goff et al., 2003) and in the mosquito *Anopheles gambiae* (David et al., 2005). Although whole genome arrays such as Affymetrix chips can detect changes in more genes (e.g. Pedra et al., 2004), practical considerations such as costs limit the number of hybridizations and hence the statistical resolving power of these arrays. Thus, "detox chips" probably should become a benchmark in the routine diagnosis of transcriptional changes in the detoxification pathways of any organism with a fully sequenced genome. Such thematic arrays have already proven their utility in vertebrate toxicology (Gerhold et al., 2001) and plant physiology (Xu et al., 2001; Kristensen et al., 2005).

Several of the genes shown to be transcriptionally regulated by phenobarbital or atrazine have been reported before to be associated with metabolic resistance to insecticides, particularly *Cyp6a2* (Waters et al., 1992; Brun et al., 1996; Amichot et al., 2004) and *Cyp6g1* (Daborn et al., 2002) but also *Cyp6a8*, *Cyp12d1*, *Cyp4e2* and *Cyp12a4* (Amichot et al., 1994; Maitra et al., 2000; Le Goff et al., 2003; Pedra et al., 2004; Bogwitz et al., 2005). Some of these genes are also inducible by other chemicals, such as DDT (Brandt et al., 2002). Insecticide resistance is generally attributed to constitutive overexpression of these genes although Amichot et al. (2004) reported that point mutations in *Cyp6a2* enable DDT metabolism. There are other well-documented cases of xenobiotic-inducible genes that are constitutively overexpressed in insecticide-resistant strains, particularly *CYP6A1* and *CYP6D1* in the house fly (review in Feyereisen, 2005). Is this relationship between induction and resistance coin-

cidental or is there an underlying mechanism that ought to be studied in greater detail? Early work, predominantly on housefly P450, had shown that resistant flies had biochemically distinct P450s, higher constitutive levels of P450, and different induction patterns. In some resistant strains induction is even greater than in susceptible strains (Terriere and Yu, 1974). In other resistant strains, P450 levels are similar to induced levels of their susceptible counterparts (or even higher) and little or no further induction can be observed (Liu and Scott, 1997). Different inducers can also be distinguished in this way, some effective in a particular strain and others not (Hallstrom et al., 1984; Zijlstra et al., 1984; Fuchs et al., 1994). Ottea and Plapp (1984) also noted that both metabolic resistance and induction were associated with the production of different forms of glutathione *S*-transferase. Terriere and Yu (1974) suggested that higher induction in resistant flies was likely due to (P450) gene amplification, but they rejected induction as a factor in the development of resistance in the field. It was suggested that the same regulatory (*trans*-acting) gene may be involved in both induction and biochemical resistance (Terriere, 1983), and Plapp (1984) further proposed that a receptor involved in xenobiotic induction may be altered in resistant insects. However, despite growing evidence that overexpression of some P450 genes in resistant strains can involve *trans*-regulating factors (e.g., Sabourault et al., 2001), a link with induction has remained tenuous. Genetic analysis in *Drosophila* indicates that expressions of *Cyp6a2* and *Cyp6a8* are controlled in *trans* by a negative regulator (Dombrowski et al., 1998; Maitra et al., 2000 and Maitra et al., 2002). If mutations alter the normal regulatory network involved in the induction of a P450 gene, this could result in higher constitutive expression of the gene and hence resistance. The link between resistance and induction would thus be coincidental. Nonetheless, genes that are readily inducible by xenobiotics have this regulatory mechanism in addition to their normal tissue, sex and developmental regulation. Thus, they offer a broader target for mutational events (point mutations, duplications, transposable element insertions, etc.) and may be more likely to be involved in resistance. Xenobiotic inducibility of a drug-metabolizing enzyme may therefore represent a risk factor for the development of resistance.

Some of the sex differences in gene expression may be attributable to sex-linked differences in metabolism and disposition of the inducers. In fact, little is known of phenobarbital or atrazine metabolism in insects (see however Natsuhara et al., 2004). Nevertheless, continuous exposure to the relatively high dose of inducer should minimize pharmacokinetic effects in favor of the pharmacodynamic effects of the chemicals.

Sex-dependent gene expression in *Drosophila* is well documented by microarray experiments (Ranz et al., 2003; Arbeitman et al., 2004) and many P450 are regulated by mating in females (McGraw et al., 2004). Male-specific constitutive expression of a P450 gene was reported previously for *Cyp312a1* (Kasai and Tomita, 2003). Our study shows that transcriptional regulation by xenobiotics is also a sex-dependent trait.

Phenobarbital is one of the most commonly used inducers of cytochromes P450, known to be effective in animals as well as plants, and even in bacteria (Fulco, 1991). For insects, induction of a P450-dependent enzyme activity (Agosin et al., 1969) and of a specific P450 gene (Cariño et al., 1992) by phenobarbital were reported long ago. Molecular studies in several insect species have now identified many phenobarbital-inducible genes (review in Feyereisen, 2005). It was already known that in the same species, several P450 genes were inducible as CYP6A1, CYP12A1 and CYP6D1 in the housefly, but here we provide a complete view of the pleiotropic effect of phenobarbital induction on the CYP family in adult fruit flies. Regulatory elements responsible for the phenobarbital response of the *Cyp6a2* and *Cyp6a8* genes of *Drosophila* are under study (Dunkov et al., 1997; Dombrowski et al., 1998; Maitra et al., 2002). Our identification of additional phenobarbital-inducible genes should facilitate this molecular study of induction.

Atrazine is another known inducer of P450. Atrazine induces P450-dependent activities and a CYP4-related P450 gene in the aquatic larvae of the midge *Chironomus tentans* (Miota et al., 2000; Londono et al., 2004). Atrazine induction of P450 activities has also been reported in *Spodoptera* larvae (Kao et al., 1995; Yu, 2004). Atrazine induced pentoxyresorufin O-dealkylation activity in rats, as well as CYP2B on western blots, in a manner similar to phenobarbital-like inducers (Ugazio et al., 1993). In *Drosophila* , we have shown that atrazine has a pattern of induction that is similar, but not identical to phenobarbital, and that is characterized by the induction of several GST genes in males. This indicates a degree of selectivity, but also an underlying complexity of the induction response. A large-scale screen of gene expression in the nematode *C. elegans* revealed that CYP31A1 and A3 are inducible by both phenobarbital and atrazine whereas CYP35A5 and 35C1 are induced by atrazine but not by phenobarbital (Menzel et al., 2001). We expect that testing more chemicals will uncover the xenobiotic regulation of different genes, and that a picture will emerge of overlapping specificities of induction of P450 genes and/or GSTs and esterases.

Closely related genes showed a differential response to the two inducing compounds: for instance, *Cyp6a19* was repressed by phenobarbital in females, but *Cyp6a2, 9, 17* and *Cyp6a23* were induced, whereas other CYP6 genes were not affected. Some genes may represent "stress-responsive genes" such as *Cyp28a5*. This gene was induced by phenobarbital in both sexes, and by atrazine in males, and this gene is also induced by paraquat, H_2O_2 and tunicamycin (Zou et al., 2000; Girardot et al., 2004). The function of the genes transcriptionally regulated by phenobarbital or atrazine is largely unknown. *Cyp6a2* is an abundant P450, enriched about 8-fold in Malpighian tubules (Wang et al., 2004) and

its ability to metabolize pesticides has been recognized (Dunkov et al., 1997; Amichot et al., 2004). *Cyp6d5* and *Cyp6w1* are represented by many ESTs and the latter was first identified in olfactory organs (Wang et al., 1999). *Cyp12d1* is of special interest because it encodes a mitochondrial P450, whereas the other genes encode microsomal P450s. The related CYP12A1 of the housefly is also inducible by phenobarbital, and metabolizes several xenobiotics (Guzov et al., 1998). The CYP12-like P450s of insects are thus a special type of inducible, environmental response genes, unlike the steroid-metabolizing mitochondrial P450s of vertebrates and insects.

Little is known of the physiological substrates of the induced enzymes but *Cyp6a2* was previously shown to be ecdysone-inducible (Spiegelman et al., 1997; see also Dunkov et al., 1997; Dombrowski et al., 1998) and to metabolize JH III and related sesquiterpenoids (Andersen et al. 1997), and *Cyp18a1* was initially cloned as a result of its ecdysone inducibility (Hurban and Thummel, 1993). Lobster CYP45, a CYP6/9-like P450 is induced by phenobarbital and by 20-hydroxyecdysone (Snyder 1998). Similarly, CYP330A1 (a member of the CYP2 clan as CYP18) is inducible by ecdysteroids and by phenobarbital in the crab *Carcinus maenas* whereas CYP4C39 is not (Rewitz et al. 2003). These genes are thus possible cross-over points between endocrine and xenobiotic signals. Interestingly, the *β*FTZ-F1nuclear receptor, down-regulated by atrazine in female flies, is known to regulate ecdysteroid titers in *Drosophila* by controlling the expression of the *dib* and *phm* genes (Parvy et al., 2005). The *dib* and *phm* genes (*Cyp302a1* and *Cyp306a1*) are expressed at levels too low to be detected in our whole-body sampling procedure. Nonetheless, the observation that yolk protein gene expression is down-regulated by atrazine as well as *β*FTZ-F1 is highly suggestive of endocrine disruption, because ecdysteroids normally induce yolk protein synthesis in female flies. Endocrine disruption is also suggested by the induction of yolk protein genes in males after phenobarbital treatment. Male flies do not normally produce vitellogenin, and until now, only 20-hydroxyecdysone excess was shown to induce yolk protein gene expression in male fat body (Bownes, 1982; Shirk et al., 1983). Xenobiotic interference with vitellogenesis has been demonstrated before in invertebrates as the functional homolog of yolk proteins, vitellogenin mRNA, is induced by bisphenol-A and vinclozolin in *C. elegans* (Kohra et al., 1999).

The exact mechanisms of these potential endocrine-disrupting effects remain to be established. For instance, we do not know yet whether the xenobiotics interact directly with hormone receptors or whether putative xenosensors such as DHR96, the *Drosophila* ortholog of PXR/CAR nuclear receptors of vertebrates, are the entry point of some xenobiotic signals. We need also to document whether xenobiotic treatments actually modify hormone levels. Xanthotoxin inducibility of the *CYP6B1* gene in the black swallowtail, *Papilio polyxenes* is dependent on a xenobiotic response element in the gene promoter. *Drosophila* transcription factors related to the vertebrate aryl hydrocarbon receptor appear to modulate expression of the *CYP6B1* promoter, but a ligand-dependent factor is not yet been identified (Brown et al., 2005). The interactions between physiological signals and xenobiotics are necessary to allow the organism to integrate information from its environment and adapt to it. The role of the so-called drug-metabolizing enzymes in this interplay is becoming clearer (reviewed by Nebert, 1991 and Nebert, 1994). Our experiments start to shed some light on the known effects of P450 inducers on insect fitness as phenobarbital treatment was shown to delay development and reduce fecundity in flies by a mechanism that has remained unclear (Yu and Terriere, 1974; Darvas et al., 1992; Fuchs et al., 1993). Further work is needed to define more precisely the subset of xenobiotic-inducible genes within the families encoding drug-metabolizing enzymes. Tests on larvae as well as adults with a greater variety of xenobiotics are thus warranted. The hypothesis that inducibility is a risk factor for resistance as discussed above will then become testable.

Acknowledgments

We thank Charles Robin, Department of Genetics, University of Melbourne for clones of most of the GSTs and esterase genes used on our array, Claire Conrad and Marcel Amichot for a set of P450 gene-specific tags, and Nathalie Tijet and Christian Helvig for their initial work on the array.

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