INVESTIGATION

Diverse Hormone Response Networks in 41 Independent *Drosophila* Cell Lines

Marcus Stoiber,*† Susan Celniker,† Lucy Cherbas,† Ben Brown,*§,1 and Peter Cherbas‡,1

*Department of Biostatistics and †Department of Genome Dynamics, University of California Berkeley, California 94720, ‡Department of Biology, Indiana University, Bloomington, Indiana 47405

ABSTRACT Steroid hormones induce cascades of gene activation and repression with transformative effects on cell fate. Steroid transduction plays a major role in the development and physiology of nearly all metazoan species, and in the progression of the most common forms of cancer. Despite the paramount importance of steroids in developmental and translational biology, a complete map of transcriptional response has not been developed for any hormone. In the case of 20-hydroxyecdysone (ecdysone) in *Drosophila melanogaster*, these trajectories range from apoptosis to immortalization. We mapped the ecdysone transduction network in a cohort of 41 cell lines, the largest such atlas yet assembled. We found that the early transcriptional response mirrors the distinctiveness of physiological origins: genes respond in restricted patterns, conditional on the expression levels of dozens of transcription factors. Only a small cohort of genes is constitutively modulated independent of initial cell state. Ecdysone-responsive genes tend to organize into directional same-stranded units, with consecutive genes induced from the same strand. Here, we identify half of the ecdysone receptor heterodimer as the primary rate-limiting step in the response, and find that initial receptor isoform levels modulate the activated cohort of target transcription factors. This atlas of steroid response reveals organizing principles of gene regulation by a model type II nuclear receptor and lays the foundation for comprehensive and predictive understanding of the ecdysone transduction network in the fruit fly.

KEYWORDS ecdysone network biology transcription RNA-seq bioinformatics

Steroid hormones provoke large, long-term effects in animals. Some are homeostatic or physiological (e.g., control of osmoregulation, immune system modulation), while others are developmental (e.g., the control of sexual maturation in vertebrates). Among the most striking of these developmental effects is the coordination of insect development and metamorphosis by the steroid molting hormone 20-hydroxyecdysone (20E, hereafter “ecdysone”). At metamorphosis, virtually every cell responds to the hormone, but the responses are as diverse as the adult organism that is being formed: many cells that played important roles during larval life are stimulated to start down pathways leading to programmed cell death, while others, destined to form adult tissues, enter pathways leading to differentiation and morphogenesis (for example, Li and White 2003). In humans, failures in steroid signaling cause diseases as diverse as mood disorders and oncogenesis. In *Drosophila melanogaster*, defects in ecdysone signaling lead to a complete failure of metamorphosis.

Hormones act by binding to nuclear hormone receptors (NHRs), which are deeply conserved across metazoans. These NHRs share a common structure: an unconserved N-terminal A/B region including a transcriptional activation domain (AF-1), followed by a highly conserved 66-68 residue DNA binding domain (DBD), a short hinge region, a conserved ligand binding domain (LBD), and unconserved sequences of variable extent called the F domain. Receptors function by binding to highly conserved response elements (HREs), where they act as powerful transcriptional effectors.

Ecdysone acts through a heterodimeric NHR composed of the products of the genes *EcR* and *ultraspiracle* (*usp*). *EcR* binds hormone...
only as part of the heterodimer (Yao et al. 1993), thus USP is an allosteric regulator with respect to ligand binding by EcR; similarly, DNA binding modifies ligand binding by the heterodimer (Azoitei and Spindler-Barth 2009). The “canonical” EcR/USP response element (EcRE) is an inverted repeat 3′-AGTCA/TGACCT-3′ (Cherbas et al. 1991), but EcR/USP also binds direct repeats and inverted repeats of diverse spacing (D’Avino et al. 1995; Braun et al. 2009; Nakagawa and Henrich 2009). EcREs are known to be present throughout the genome (Cherbas et al. 1988; Koelle et al. 1991; Yao et al. 1993).

Numerous EcR/USP coregulators have been identified. Davis et al. (2011) carried out a bioinformatic search looking for potential coregulators based on the LXXLL motif common to many hormone receptors. Triithorax-related (TRR) is known to interact with EcR/USP and to methylate H3K4 (Sedkov et al. 2003). Cryptopheal (Drosophila ATF4) is known to interact directly with isoform B2 (Gauthier et al. 2012), Taiman (TAL), a p160 homolog, and Alien, a corepressor, colocalize with the receptor (Dressel et al. 1999; Nakagawa and Henrich 2009). There is evidence implicating the products of Trig, Ash2, βFtz-F1, and the histone chaperone DEK as coregulators or critical components of coregulator complexes (Zhu et al. 2006; Sawatubashi et al. 2010; Carbonell et al. 2013). Drosophila SMRTER (Smr, a relative of SMRT and NCoR) is known to be crucial to ligand-independent reception (Tasi et al. 1999; Sedkov et al. 2003). There is ample evidence that remodeling factors, including SWI/SNF and the NURF complex, interact with EcR/USP and play key roles in ecdyson response (Badenhorst et al. 2005; Zraly et al. 2006; Ables and Drummond-Barbosa 2010; Kugler et al. 2011; Zraly and Dingwall 2012). There is also evidence that ecysone-induced expression is associated with acetylation of H3K23 (Bodai et al. 2012).

Typically, steroids induce (and/or repress) a limited number of direct coding and noncoding target genes including transcription factors (TFs) and microRNAs (Garbuzov and Tatar 2010). These responses ramify within hours, leading to secondary effects that may implicate thousands of genes and major changes in cell state. This pattern holds true for the ecysone response; indeed, some of the earliest studies of primary and of genes and major changes in cell state. This pattern holds true for the ecysone response; indeed, some of the earliest studies of primary and

MATERIALS AND METHODS

Cell lines and ecysone treatment
The individual cell lines are listed in Table 1. All cell lines used in this study were obtained from the Drosophila Genomics Resource Center (DGRC, dgrc.cgb.indiana.edu), and grown as recommended there. Each cell line used in this paper has a formal name, which is the unique identifier used by FlyBase and the DGRC. For convenience, we use a shortened version of many of these cell line names in this paper; both the formal names and the short names are given in Table 1. All of the cell lines were of independent origin. S2 cells have been grown for many years in a large number of laboratories, under variable conditions, and different isolates from the same original line (Schneider 1972) often have widely different properties (Y. Zou and L. Cherbas, unpublished results); two isolates of largely unknown provenance (S2-DRSC and Sg4) were used in the experiments reported here. Kc was also grown in multiple laboratories for a number of years after it was cloned. All of the other lines were obtained by the DGRC from the laboratories in which they were established and can be assumed to have been maintained carefully with a limited number of transfers. All experiments were performed on cells in exponential growth.

Cells were treated with 20-hydroxyecdysone (10−6 M final concentration, Sigma-Aldrich) as described previously (Savakis et al. 1980). RNA was extracted with Trizol (Invitrogen) according to the manufacturer’s
protocol; in a few cases the RNA was further purified on RNeasy columns (Qiagen). A full description of the procedure can be found in (Eads et al. 2006). Samples were sequenced on Illumina Hi-seq machines and produced single-end, unstranded, 100 bp reads.

**Differential expression analysis**

Gene and exon level counts were computed using the python package HTSeq (Anders et al. 2015) (version 0.6.1p1) using the FlyBase annotation version r5.57 (St Pierre et al. 2014). Exon level counts were analyzed using the DEXSeq R package (Anders et al. 2012, version 1.12.1). Exon level ecdysone exposure effects are reported only for a model fit across all cell lines. Thus, exon p-values and fold changes are not reported for each cell line individually. Gene level analysis was completed using the DESeq R package (Anders and Huber 2010, version 1.18.0). As only a portion of the samples were completed in biological duplicate, gene level dispersion estimates were made using the replicated samples and applied to all cell lines. The statistical assumption underlying this analysis is that gene-level biological dispersion is consistent across cell lines. Reported log2 fold change and p-values are reported in Supporting Information, Table S5.

**Identification of widespread and restricted ecdysone-responsive genes**

In order to leverage the breadth of cell lines examined in this study while identifying ecdysone-responsive genes, we employed a threshold based...

---

**Table 1 Cell lines used in this study**

| Short Name of Cell Line | Formal Name | Tissue of Origin | Ref. | Features | Total Read Depth (M) |
|-------------------------|-------------|------------------|------|----------|---------------------|
| 1182-4H                 | 1182-4H     | Embryo           | 1    | FCB      | 136.15              |
| CCa                     | CCa         | Embryo           | 2    | MD       | 210.38              |
| L1                      | CME L1      | Prothoracic leg disc (L3) | 3   | MVCBI    | 108.1               |
| Cl.8                    | CME W1 Cl.8* | Wing disc (L3)  | 3   | MVC1     | 131.06              |
| W2                      | CME W2      | Wing disc (L3)   | 3   | MVCBI    | 107.09              |
| D1                      | D1          | Embryo           | 4    | M        | 83.76               |
| DX                      | DX          | Embryo           | 2    | A        | 148.5               |
| E-CS                    | E-CS        | Embryo           | 5    | F        | 146.78              |
| E-OR                    | E-OR        | Embryo           | 5    | M        | 123.98              |
| G1                      | G1          | Embryo           | 6    | M        | 95.83               |
| G2                      | G2          | Embryo           | 6    | M        | 140.4               |
| GM2                     | GM2         | Embryo           | 7    | MCB      | 142.59              |
| GM3                     | GM3         | Embryo           | 7    | MCB      | 91.36               |
| Jupiter                 | Jupiter     | Embryo           | 8    | M        | 63.53               |
| Kc                      | Kc167       | Embryo           | 9    | FVCBDIE  | 197.17              |
| mbn2                    | mbn2        | Hemolymph (L3)   | 10   | MCB      | 62.92               |
| MCV12                   | MCV12       | Wing disc (L3)   | 11   | FVD      | 143.57              |
| MLB3-26                 | MLB3-26     | Embryo           | 12   | F        | 123.78              |
| BG1-c1                  | ML-DmBG1-c1 | CNS (L3)         | 13   | AVCB     | 114.13              |
| BG2-c2                  | ML-DmBG2-c2 | CNS (L3)         | 13   | MVCBI    | 147.65              |
| BG3-c2                  | ML-DmBG3-c2 | CNS (L3)         | 13   | MVCBI    | 320.94              |
| D1-c4                   | ML-DmD1-c4  | Wing disc (L3)   | 14   | MV       | 80.78               |
| D11                     | ML-DmD11    | Eye-antennal disc (L3) | 14  | MCB      | 188.04              |
| D17-c3                  | ML-DmD17-c3 | Haltere disc (L3) | 14  | FVCB     | 86.2                |
| D20-c5                  | ML-DmD20-c5 | Antennal disc (L3) | 14  | MCB      | 183.64              |
| D21                     | ML-DmDm21   | Wing disc (L3)   | 14   | MCB      | 105.42              |
| D23-c4                  | ML-DmD23-c4 | Wing disc (L3)   | 14   | MV       | 98.39               |
| D4-c1                   | ML-DmD4-c1  | Mixed imaginal discs (L3) | 14  | MCB      | 147.7               |
| D8                      | ML-DmD8     | Wing disc (L3)   | 14   | FVCB     | 223.87              |
| D9                      | ML-DmD9     | Wing disc (L3)   | 14   | AVCB     | 194.2               |
| OSS                     | OSS         | Ovary (Adult)    | 15   | FVPB     | 113.75              |
| PR-8                    | PR-8        | Embryo           | 16   | M        | 173.2               |
| Pten X                  | Pten X      | Embryo           | 16   | M        | 210.2               |
| Ras-wts:RNAi            | Ras[v12];wts[RNAi] | Embryo | 17   | M        | 105.12              |
| Ras-H3                  | Ras[v12]-H3 | Embryo           | 18   | M        | 163.44              |
| Ras-H7                  | Ras[v12]-H7 | Embryo           | 18   | M        | 189.44              |
| Rumi-Ras                | Rumi[26];Ras[v12]-4 | Embryo | 19   | FR       | 119.24              |
| S1                      | S1          | Embryo           | 20   | MCB      | 126.57              |
| S2-DRSC                 | S2-DRSC     | Embryo           | 20   | MCE      | 102.67              |
| S3                      | S3          | Embryo           | 20   | MCB      | 123.66              |
| Sg4                     | Sg4         | Embryo           | 20   | MCB      | 85.86               |

References: 1, Debec 1978; 2, V. Gvozdev, personal communication; 3, Currie et al. 1988; 4, A. Dubendorfer, personal communication; 5, Bernhard et al. 1980; 6, W. Gehring, personal communication; 7, Mosna and Dolfini 1972; 8, Karpova et al. 2006; 9, Echalier and Ohanessian 1969; 10, Gateff et al. 1980; 11, M. Milner, personal communication; 12, T. Miyake, personal communication; 13, Uri et al. 1994; 14, Uri et al., 1987, 15, Niki et al. 2006; 16, Justinianno et al. 2012; 17, Simcox et al. 2008; 18, Dequeant et al. 2015; 19, Leonardi et al. 2011; and 20, (Schneider 1972). Features: F, female; C, transcriptome described in Cherbas et al. 2011; B, transcriptome described in Brown et al. 2014; M, male (M, F, or A assignment from Lee et al. 2014 or by the same criteria using data from this paper; A, ambiguous gender; P, the parental line FGS/OSS is a coculture of somatic sheath and germ cells (OSS was generated from FGS/OSS by the loss of the germ cell component (Niki et al. 2006)); D, duplicated in RNA-seq analysis; V, variation to standard medium (M3+BPYE with heat-inactivated fetal calf serum, particular variations are noted in Table S1); I, analyzed by microarray in preliminary ecdysone study; E, analyzed in extended ecdysone response time course (0, 1, 3, 5, and 7 hr); R, cell line expressing constitutively active Ras85.
on the responsiveness across all cell lines. This threshold allows genes that show a trend toward significance, while possibly not achieving standard statistical significance within any particular cell line, to be confidently identified as ecdysone-responsive. Formally, this is measured by the Fisher’s Method test for trend in significance across all cell lines.

Each gene is associated with two Fisher’s Method values corresponding to a trend toward induction and repression across all cell lines. We note that it is possible to achieve significance in both responsive behaviors under this structure. The procedure to produce these tests is analogous for induction and repression, so we will describe the procedure for induction here. In order to compute a Fisher’s Method significance value, the p-values produced by the differential expression analysis are used to construct a ranked list of induced genes within each cell line. Genes that are repressed are assigned a p-value of one and thus are tied at the bottom of that cell line’s rank list. These rank values thus represent a uniform marginal distribution for each cell line, as required in order to apply Fisher’s Method. For each gene, the rank within each cell line is combined using Fisher’s Method. Genes that tend toward the top of the rank list in many cell lines will produce significant values, while genes randomly distributed among each list will produce less significant values.

Two types of thresholds, biological relevance and statistical significance, are applied to each gene within each cell line. The biological relevance threshold is defined by a fold change upon ecdysone exposure greater than twofold (inductive or repressive). Statistically significant responsive genes are those that achieved either an adjusted p-value less than 0.01 regardless of Fisher’s Method p-value, or an unadjusted p-value of 0.01 and a Fisher’s Method p-value less than $10^{-8}$. These genes identified as significantly ecdysone-responsive in a particular cell line can be found in Table S6.

**Enrichment of motifs**

Enrichment of motifs within promoter region DNA sequences was carried out using the homer2 program (Heinz et al. 2010) with the “known” command against the supplied all.motifs database, which contains the EcRE motif of interest. Scripts and database are available online http://homer.salk.edu/homer/motif/.

**Summary analysis**

All statistical analyses were computed using R (version 3.1.2) using custom scripts. Gene lengths for length-normalized expression were taken as the mean of the lengths of the transcripts for that gene. GO term enrichment was produced using the fb_2014_03 version of the FlyBase gene ontology (Gene Ontology et al. 2013). Only genes with at least one annotated ontology term were used for enrichment calculations. All GO term enrichment p-values were calculated using the hypergeometric distribution.

**Responsive proximal genes**

For all significantly responsive genes, the fraction of responsive genes and the average response direction of nearby genes were analyzed. In order to determine the distance between two genes, the ecdysone relevant transcription start sites (TSS) were first determined. For genes with multiple transcription start sites, the TSS was determined to be the TSS associated with a significant exon level response to ecdysone if one exists, or the exon with the highest length normalized expression in the relevant time point (i.e., 5 hr time point for induced genes, 0 hr time point for repressed genes, or the average for nonresponsive genes). The selected transcription start sites are included in Table S5.

Each gene within 20,000 base pairs of a responsive gene was associated with two values, first if the gene was responsive and second the direction of response, taken as the negative log10 of the p-value multiplied by the sign of the log fold change after ecdysone exposure divided by before (these values are trimmed to plus and minus 10 to avoid outlier effects). A moving window of 100 gene–cell line combinations was used to calculate the fraction of responsive genes and the average response direction within each bin. The binned points were grouped according to the response direction of the responsive gene as well as the shared promoter architecture (upstream/downstream and same/opposite strand). These points were then smoothed using LOESS with a local linear fit over binned points. The plot produced is found in Figure 3.

**Restricted ecdysone-response prediction**

In order to predict the restricted response, the following two models (referred to as matched induction and matched repression) were fit. The models are symmetric, so only the matched induced model will be described in full detail.

At each gene in the restricted induced set, we aimed to predict which cell lines responded significantly and which responded most repressively, defined as the cell lines showing the smallest log2 fold change of ecdysone exposure expression/preexposure expression. A matching number of the most repressive responsive cell lines were chosen such that each gene contained a balanced number of induced and matched repressed cell lines.

In order to predict which gene–cell line combinations belonged to the above described sets, the model was provided with normalized TF expression masked by a known TF motif from the TOMTOM database (Gupta et al. 2007), present in the promoter of the gene to be predicted. TF expression was included only if at least one cell line expressed the TF above the 20th percentile of genes with at least one read. A TF motif was considered significant by setting the threshold on the allowed mismatches to the known PWM, such that just less than 5% of nonresponsive genes’ promoters contained a hit to the motif. There were 270 TFs with known motifs and valid expression. Additionally, the model was given the rank of the normalized expression of the gene to be predicted, as lowly expressed genes are intuitively less likely to be repressed and more likely to be induced, and conversely for highly expressed genes.

This model was then fit using the random forests model (Breiman 2001; Abraham et al. 2014). Important variables were determined from a model fit on all 41 cell lines. Accuracy measures were obtained by constructing the above outcome and predictor variables and then removing each cell line as a test set. The data from the remaining cell lines were used to train the random forest and the data from the left out cell line was used to test the accuracy of the model’s predictions. Accuracy measures from all cell lines were averaged and reported.

In order to determine the effect of increased numbers of cell lines on prediction accuracy, samples of cell lines were taken randomly and restricted responding genes, outcome, and predictor data were constructed. The left out cell line method was again used to determine the accuracy of the model. Since there were many subsets of cell lines that could be chosen, the subsetting procedure was repeated 1000 times for each number of cell lines and all average accuracy values are reported.

**Extended time course analysis**

For a subset of three cell lines (Kc, BG3, and S2) that have an extended time course, including the 0, 1, 3, 5, and 7 hr time points, the following analysis pipeline was conducted. In order to compare the transcriptional responses across the time course for each cell line, a normalization was performed that allows comparison of genes with dissimilar steady state expression levels, but may share ecdysone response “shape.” This normalization begins by applying the robust median library size
normalization from the DESeq R package (Anders and Huber 2010). Then, a mean centering is applied at each gene and cell line across all five time points. The gene–cell line expression is then divided by the fitted standard deviation across all gene–cell line combinations, in order to adjust for the known increase in variance at higher expression loci. These normalized expression measures can thus be interpreted as a response shape across time for each cell line and the response shape is comparable for genes at different mean expression levels, but the trend and relative scale of response over time is maintained. Units represent standard deviations from the mean across time. These normalized expression values are analyzed in the context of the response at the 5 hr time point.

**Data availability**

All sequences are available through the SRA (BioProject PRNA306537) and individual sample accession numbers are available in Table S2 Microarray data re in the GEO database at NCBI as series GSE11167.

**RESULTS**

**Overview of study design**

RNA samples were collected from 41 cell lines (Table 1 and Table S1) before and after a 5 hr exposure to ecdysone at a biologically relevant concentration of $10^{-6}$ M. Transcription levels were measured by single-end poly(A)$^+$ RNA-sequencing with 100 bp reads. For four of the cell lines, Cca, Kc, MCW12 and BG3-c2, duplicate samples were collected in order to estimate the biological variation present in this system. Samples were taken from an extended time course of exposure at 1, 3, 5, and 7 hr for three cell lines, Kc, BG3-c2 and S2-DRSC. All sequences are available through the SRA (BioProject PRNA306537) and individual sample accession numbers are available in Table S2. Gene and exon-level transcriptional quantifications were assessed using the FlyBase r5.57 annotation (Table S3 and Table S4). Differential expression calculations were carried out using the DESeq (Anders and Huber 2010) R package (see **Materials and Methods**). Significantly induced or repressed genes were identified by applying a biological relevance fold change threshold of two and a statistical significance threshold of 0.01 adjusted p-value. A relaxed statistical significance threshold of 0.01 unadjusted p-value was applied for genes that showed a strongly significant tendency for induction or repression across many cell lines (Fisher’s method p-value $< 10^{-8}$, see **Materials and Methods**). Differentially expressed (DE) genes along with meta-information can be found in Table S5 and Table S6. (Note that Table S5 is interactive and enables the exploration of the effects of alternative thresholds.)

Additionally, microarray experiments examined 12 cell lines (control and 5 hr after ecdysone treatment) using DGRGC-2 microarrays (oligonucleotides representing 64,020 exons from 14,518 genes as annotated in FlyBase v 2010-02). Effects were scored as significant at an FDR threshold of 1%. All results have been deposited in GEO. Data were biologically replicated in triplicate and results can be found in Table S5 along with matched RNA-seq results.

**Diverse cell states profiled**

Cell lines are remarkably transcriptionally diverse (Cherbas et al. 2011). More than 70% of all *Drosophila* genes (11,884) are expressed at greater than one RPKM (reads per kb per million mapped reads) in at least one of the 41 cell lines we profiled at ground state (prior to ecdysone exposure). Of those, 5846 are constitutively expressed in all cell lines, while 1459 are expressed in only a single cell line. The number of expressed TFs per cell line ranges from 406 to 450, and 595 (85% of all TFs in fly, Hammonds et al. 2013) are expressed in at least one cell line. Further, TFs are expressed at quantitatively different levels in each cell line (Figure S1). After ecdysone exposure, an additional 305 genes are expressed that were not basally detectable in any cell line and, conversely, 360 genes are constitutively inactivated after ecdysone exposure. We defined genes with significant responses in more than half of the cell lines as “widespread,” and others as “restricted.”

The vast majority of restricted genes are expressed in only a few cell lines; only 100 comprise the widespread class (Figure 1, A and B). Indeed, few pairs of cell lines overlap in their restricted responses by more than 20% (Figure 1C and Figure S2). The two most striking clusters of cell lines (cluster 1: D8, BG3-c2, D23-c4, D4-c1, Ras-H7, and Jupiter, and cluster 2: D11, E-OR, D21, ML83-26, and MCW12; emphasized in Figure 1 by red boxes) do not share physiological origin, sex of the cells, or other covariates as seen in Figure S3. In general, the response to ecdysone across these cell lines does not appear to be well correlated with common cell line characteristics. Therefore, this in vitro system provides the opportunity to study diverse and distinct ecdysone response dynamics as a function of initial transcriptomic cell states.

We note that the collection of cell lines includes six created by expression of a constitutively active Ras oncogene (Ras-H3, Ras-H7, Ras-wtsRNAi, and Rumi-Ras), a null mutation in the tumor suppressor gene Pten (PtenX), or both (PR-8) (Simcox et al. 2008; Justiniano et al. 2012). Four of these cell lines cluster when ecdysone responses are compared (Figure 1 and Figure S3). An additional activated Ras line (Ras-H7) shows some similarity in its responses. If confirmed, the existence of a Ras/Pten cluster could suggest that the pathway to generation of a cell line by either gain of Ras function or loss of Pten function leads to a transcriptional environment conducive to a particular pattern of ecdysone response.

**Ecdysone receptor is rate-limiting for global responsiveness**

A total of 1645 genes are significantly transcriptionally responsive in at least one cell line. Fifty-nine TFs are induced in response to ecdysone, and 35 of these are responsive in five or fewer cell lines (Table S5). Several of these are known ecdysone-responsive TFs, while many are newly identified and point to new hormone-responsive pathways. Table S7 provides enriched GO terms among induced and repressed genes.

It is well known that some tissues and cell types are more responsive to ecdysone than others. We measured the responsiveness of a cell line as the count of genes significantly induced or repressed 5 hr after induction, and refer to this as the Responsive Gene Count (RGC). While RGC is threshold-dependent, the rank-order of cellular responsiveness is well preserved across a broad range of biologically and statistically meaningful parameterizations (see **Materials and Methods**, Table S8). The RGC varies by two orders of magnitude across cell lines and is driven by responsive genes with highly restricted expression patterns (Figure 1 and Figure S4).

The RGC does not correlate with the count of basally expressed TFs ($r \sim 0.04$, $p = 0.85$) and correlates only weakly (and inversely) with the total number of genes expressed per cell line ($r \sim 0.35$, $p = 0.03$). We assessed the association of the RGC with the basal expression level of each gene in the genome in a multiple testing setting (see **Materials and Methods**). Among all genes, the expression level of EcR is by far the most strongly correlated with RGC ($r \sim 0.71$, $FDR < 0.001$, minimum FDR for other genes $> 0.03$, Table S8). We further assessed this observation within both induced and repressed genes (Figure 2) and found that EcR expression level is strongly correlated with both. In fact, EcR is the only gene statistically significantly correlated with the number of both induced and repressed genes (EcR $FDR < 0.001$, minimum FDR for other genes $> 0.02$). The EcR heterodimer
partner, usp, exhibits weak correlation with RGC ($r > 0.35$, FDR $> 0.9$; Figure 2).

The residuals left after correcting for the effect of $E_{CR}$ expression level on RGC, as well as on the counts of induced and repressed genes (separately), show little correlation with any gene (maximal correlation $< 0.61$; minimum FDR $> 0.6$). Taken together, these results indicate that $E_{CR}$ titer is rate-limiting for the transcriptional response to ecdysone.

**Figure 1** Cell line responses: breadth and similarity. Using the thresholds defined in the text, the inductive (A) and repressive (B) response within each cell line is represented. The red shaded bars represent widespread genes (responsive in more than half of cell lines) and black shaded bars indicate restricted response genes (responsive in less than half of cell lines), as noted in the legend. Main histograms show the response of each cell line, ordered by total number of responsive genes $E_{OR}$, Ras-H3, D11, BG3-c2, D23-c4, D21, D8, MCW12, Sg4, S3, ML83-26, PR-8, D4-c1, D20-c5, G1, Rumi-Ras, Pten X, Jupiter, Ras-H7, GM3, D1-c4, 1182-4H, S2-DRSC, S1, CCa, W2, L1, D9, E-CS, DX, CI.8, Kc, mbn2, BG1-c1, GM2, OSS, D1, D17-c3, BG2-c2, G2, and Ras-wts:RNAi. Inset histograms show the number of cell lines in which each gene is responsive. (C) Cell line similarity (Jaccard similarity) within the restricted response is used to cluster the cell lines as shown in the dendrogram on the left. Repressive (lower left) and inductive (upper right) response similarity corresponds to the scale indicated on the lower right. Stacked barplots in C are the same as those in A and B, reordered by the hierarchical clustering dendrogram into the following order (from left to right): D11(1), E-OR, D21, ML83-26, MCW12(5), Pten X, Ras-H3, Rumi-Ras, PR-8, 1182-4H(10), D20-c5, D1-c4, GM3, S1, S3, D8, BG3-c2, D23-c4(20), D4-c1, Ras-H7, Jupiter, D9, W2(25), E-CS, S1, GM2, Kc, DX(30), CI.8, L1, CCa, mbn2, Ras-wts:RNAi(35), G2, D17-c3, OSS, D1, BG2-c2(40), and BG1-c1.
Common ecdysone response: the widespread set
The 100 genes with widespread responses include 68 induced and 32 repressed in more than half of our cell lines. Five genes, Hr4, Hormone receptor-like in 46 (Hr46), Ecdysone-induced protein 75B (Eip75B), CG4004, and bip1, are induced in all 41 cell lines. All five have been previously identified in other genome-wide surveys of ecdysone exposure response (Reckstead et al. 2005; Gonsalves et al. 2011; Gauhar et al. 2009; Shlyueva et al. 2014) (Table S10). There are no genes repressed in all cell lines, with fruitless (fru) being the most widespread (repressed in 33 of 41 cell lines). More broadly, widespread induced genes are significantly enriched for biological GO terms “metamorphosis,” “salivary gland cell autophagic cell death” and “steroid hormone mediated signaling” (p-value < 0.001), as expected, since the majority of these genes have been previously reported in other studies of ecdysone response (Table S10).

The promoter regions of widespread induced genes, defined as 500 bp upstream of each TSS, are 36% enriched over background for the EcR motif (p-value < 0.02), representing the most significantly enriched of all known motifs in the HOMER library (Heinz et al. 2010) (see Materials and Methods).

Notably, 11 widespread induced genes lack GO annotations, and these include four annotated long noncoding RNA (lncRNA) genes, CR43432, CR43626, CR45391, and CR45424. These lncRNAs are each expressed in the salivary gland and fat body, and at low levels in most other tissues. Only CR43432 is expressed at high levels during development, with maximal expression (> 100 RPKM) in the 4–14 hr embryos, consistent with response to the midembryological ecdysone pulse. This is in contrast to the majority of lncRNAs in Drosophila (and indeed mammals), which are expressed predominantly in tissues of the nervous system and the gonads (Brown et al. 2014; Derrien et al. 2012). Notably, this lncRNA is induced at levels comparable the well-known response polished rice, which encodes short (11 amino acid) peptides critical for ecdysone transduction in the epidermis (Chanut-Delalande et al. 2014). CR43432 encodes three short, ultraconserved ORFs, and hence constitutes a candidate protein-coding gene.

The set of widespread repressed genes is much smaller than the set of induced genes, as is the overall repressive response in most cell lines. There are no statistically enriched GO terms among this set of genes.

Diversity of the ecdysone response: the restricted set
We find that 93% (863) of induced genes and 96% (756) of repressed genes are affected by ecdysone in fewer than half our cell lines. These restricted responses form the molecular basis of the diverse transcriptional and physiological effects that the hormone induces throughout development and within distinct cell types. A total of 400 and 241 genes are induced and repressed, respectively, in exactly one cell line, with a large fraction (31%) responding only in the Ras-H3 cell line, an outlier in this study (see Materials and Methods).

Eighty-three genes are significantly induced in some cell lines and significantly repressed in others (Table S11). Sixteen are induced and repressed in at least two cell lines. One striking example is the TF CG9932, which is significantly induced in five cell lines and significantly repressed in six. CG9932 is differentially expressed across development with peaks at 20 hr and late L3 stage and shows strong expression in embryonic fat body and salivary glands. It is likely that some promoters respond in distinct directions based on prior epigenetic state. This phenomenon has also been noted in mammalian response to glucocorticoids (Chodankar et al. 2014).

The restricted set includes genes that respond weakly in several cell lines. Some of these genes do not pass our statistical criteria in any single sample, but by aggregating information across cell lines we obtain sufficient power to confidently annotate weak, reproducible induction or repression (see Materials and Methods). A total of 635 genes, 335 induced and 300 repressed, of this type are present genome-wide. Hence, leveraging our large number of cell lines we are able to confidently detect weak modulation of a large cohort of genes. These relatively small perturbations may be of no physiological significance to the cell; however, this may not always be the case. Weakly induced genes are strongly enriched for GO terms including “protein binding,” “vesicle-mediated transport,” and “macrophagy,” and weakly repressed genes are enriched for “rRNA processing” and “calmodulin binding” (Table S7). Indeed, all five core TFIIH complex genes (and all nine components except hay) are weakly repressed in at least two-thirds of cell lines, while not a single gene is significantly responsive. While each of these individual modulations is small, the cumulative effect in a given cell on the titer of the functional TFIIH complex may be substantial. Genome-wide, weakly induced genes across most cell lines are found within 2.5 kb of a significantly induced gene more than expected...
by chance (84% enrichment, binomial p-value $< 10^{-5}$). This pattern does not hold for weakly repressed genes.

**Responsive–proximal genes tend to respond similarly**

Genes proximal to responsive genes tend to be responsive (p-value $< 10^{-15}$, sample t-test against median for genes between 10–20 kb) and additionally tend to respond in the same direction (p-value $< 10^{-100}$ induced and p-value $< 10^{-19}$ repressed; one-sample t-test). The response of opposite-strand gene pairs (bidirectional and convergent) is less coordinated than genes with operon-type architecture (same strand pairs, Figure 3A and Figure S5, p-value $< 10^{-10}$). One striking example of a divergently responsive bidirectional pair is Piezo and CG8498. The TSSs of these genes are separated by only 655 bp (Figure 3B) and the responses in most cell lines are strong and in opposite directions. CG8498 is a widespread induced gene with 38 cell lines responding significantly, and all but two cell lines showing repression of Piezo. One example of co-responsive genes in an operon-type configuration is the pair CG43389 and the noncoding gene CR43626, which are significantly induced in 32 and 35 cell lines, respectively (Figure 3C).

Taken together, these results indicate that response to ecdysone may involve or depend upon local chromatin organization or modification. Indeed, the gene encoding the bromodomain protein toutatis (tou), a protein involved in chromatin remodeling (Vanolst et al. 2005), is strongly induced in 15 cell lines. Both an acetylysine binding domain (bromodomain) and a methyl-CpG binding domain exist in TOU. As a class, these BAZ (bromodomain adjacent to zinc finger) genes appear to be involved in the integration of information encoded in DNA methylation and posttranslational histone modifications (Filippakopoulos and Knapp 2014). The involvement of acetyllysine binding factors is consistent with previous reports demonstrating that ecdysone transduction impacts H3K23 acetylation (Bodai et al. 2012).

**Isoform-specific ecdysone responses**

Along with the gene level response to ecdysone we observe induction and repression of specific exons/isoforms in response to ecdysone, predominantly promoter-switching events, as has been previously reported (Shlyueva et al. 2014). The widespread responsive gene Eip75B shows the strongest exon level event in the genome across all cell lines (p-value $< 10^{-100}$), consistent with previous reports (Bernardo et al. 2009; Shlyueva et al. 2014). Sequencing tracks and exon level expression analysis are found in Figure S6 and Figure S7. In total, 35 genes show significant exon level induction events and 31 genes show significant repression events (thresholds: adj. p-value $< 0.01$ and 50-fold change). Six genes, including previously reported events at Eip75B and Eip74EF and novel events at Ect, show significant induced and repressed exons representing promoter-switching events. Exon level statistics for significant events are given in Table S12.

**Ecr isoforms**

Cherbas et al. (2003) demonstrated that the alternative Ecr isoforms (Ecr-A, consisting of transcripts A, D, and E; and Ecr-B1/2, consisting of transcripts B, C, and G) play important roles in development.
Gonsalves et al. (2011) showed that EcR isoforms are differentially expressed between Kc cells and salivary gland cells, indicating that alternate EcR isoforms elicit different transcriptional responses. Cell lines in this study show a wide range of EcR isoform expression. The length normalized fraction of the alternate expressed between Kc cells and salivary gland cells, indicating that lines in this study show a wide range of cell lines expressing predominantly the EcR-B1/2 isoform. We do not see a strong correlation to the total number of induced or repressed genes, or the residuals after correcting for the main EcR expression effect, but we do observe strong correlation between the EcR-B1/2 isoform fraction and the expression of many individual genes.

The most significantly correlated genes are gliolectin (glec), squeeze (sqt), CG5059, Eip55E, and broad (br) (top 100 genes are listed in Table S13). Only glec and Xbp1 show increased expression with increased EcR-A isoform levels among the top 10 most correlated, consistent with a predominantly repressive role for EcR-A (Wilcoxon rank-sum p-value < 10^{-10}). Of the highly correlated genes, the TF br shows the largest dynamic range (two orders of magnitude). Twenty-three cell lines show significant induction and one, BG3-c2, shows significant repression (Figure 4).

**Restricted responses depend in detail on the expressed cohort of TFs**

While the global level of responsiveness (ERG) is well characterized by EcR titer, other effects are clearly at work producing diverse restricted responses. TFs are likely candidate EcR cooperative factors. We developed a statistical machine-learning model aimed at identifying these factors: we used basal expression levels and TF binding motifs to predict restricted responses. We also supplied the model with information about the basal expression level of each gene, since it is more difficult to detect repression of genes that are poorly expressed, and to detect induction of genes that are highly expressed.

We fit models for induction and repression. A notable feature of our modeling strategy is that we hold out entire cell lines, so when we assess our model’s predictive performance, it is assessed on cell lines it has not previously seen. This ensures that our model is generalizable to unseen cell lines. We find a predictive accuracy on held-out cell lines of 61% and 64% for induced and repressed genes, respectively, indicating that we have weak but significant power to predict the direction of a gene’s response.

We used feature selection to compute the relative importance of each covariate in our model (see Materials and Methods). We find that, for models of both induction and repression, the rank of basal gene expression level is the most important covariate. For the repression model, we see that gene rank is two and half times more important than the most important TF. In the induction model, the gene rank is only one and one-third times more important. However, in both models, several TFs also show comparably significant importance values (listed in Table S14). The most important TFs include known ecdysone response factors br and Eip74EF. TFs not previously implicated in the ecdysone response are also important; these include longitudinal lacking (lola) and Chorion factor 2 (Cf2). We note nearly all covariates have weak positive values, indicating that the restricted response is a function of a large number of TFs, and hence that ecdysone response depends in detail on the expressed cohort of TFs.

This approach also enables us to assess the power of transcriptional profiling for elucidating the basis of hormone responses. We fitted and assessed this model successively using different numbers of cell lines, between 4 and 40 (Figure S8). From this analysis, we extrapolated a theoretical maximal accuracy (asymptote) for identifying the response of an unseen cell line of 74% and 79%, respectively, given an infinite number of cell lines in the training set (see Materials and Methods). Therefore, transcriptional profiling alone is not sufficient to fully elucidate the ecdysone response.

**Dynamics of extended temporal response**

In addition to the response detected at the 5 hr time point, we explored the response to ecdysone for an extended temporal range, including 1, 3, 5, and 7 hr after exposure for three cell lines, BG3-c2, Kc and S2. We normalized responses, setting the basal expression level of each gene to zero, and then quantified changes at subsequent time points in multiples of fitted standard deviations (see Materials and Methods). This intuitive representation captures much of the same information as z-scores, and has the advantage that each gene is set to the same (zero) value in basal conditions. We used this representation to identify structures in both the scales and directionalities of temporal responses (Figure 5).

Among genes that show significant induction in all three cell lines, genes in the BG3-c2 cell line respond systematically more rapidly. Expression levels in this cell line also level off more quickly than in S2 and Kc, which show steadily rising expression through all 7 hr.

There are 15 genes that are responsive at 3 hr after induction only in Kc and S2 cells but not in BG3-c2, and these show a strong and consistent expression pattern in the BG3-c2 time course: they are responsive at 1 hr, and then reduce expression level at each time point thereafter. Six of these 15 genes are widespread responsive (p-value < 1e^{-10}), including br and Eip71CD. The rapid (at 1 hr) induction of these genes indicates that BG3-c2 is in a more ecdysone sensitive basal state. Notably, the total responsiveness of these three cell lines differs substantially: the RGC value for BG3-c2 cells is more than twice that of S2 or Kc cells. Taken together, these data indicate that the observed increased responsiveness of BG3-c2 cells at 5 hr may be due to an accelerated ecdysone response relative to other cells.

**DISCUSSION**

Leveraging the intrinsic variation in initial state among cell lines, we report the most expansive catalog to date describing the ecdysone response. Our observation that the overall responsiveness of a cell line to ecdysone correlates strongly with EcR mRNA titer indicates that the availability of EcR is the primary rate-limiting step in the ecdysone response. Further, we find that EcR isoform titer is a powerful predictor of response direction and magnitude for several genes including br, a
responses must re

genes regulated in only one or a few cell lines. The diversity of these
suggests a role for Pol2-associated chromatin modi-
resolution of ecdysone transduction along the genome is on the order of
dependent control. This is remarkable and indicates that the spatial
and a number of bidirectional promoters are found to be under in-
secutive proximal gene pairs preferentially organize on the same strand
investigating.

These genes are of special interest because understanding how their
siveness (positive, negative, or null) varies among the panel of lines.
we have identi-
strongly modulated genes and includes many genes previously identi-
alyzed in genetic and biochemical studies of the ecdysone response. In
numbers, the far larger set, our “restricted” responders, represents
genes regulated in only one or a few cell lines. The diversity of these
responses must reflect varying chromatin, transcription factor, small
RNA, and other conditions present in the individual cell lines. Among
these, we have identified a substantial number of genes whose respons-
iveness (positive, negative, or null) varies among the panel of lines. These genes are of special interest because understanding how their behaviors are dictated by cellular state encapsulates the problem we are investigating.

The organization of responsive genes in the genome supports the idea
that epigenetic state, including chromatin context, is prominent. Re-
sponsive proximal gene pairs preferentially organize on the same strand
and a number of bidirectional promoters are found to be under in-
dependent control. This is remarkable and indicates that the spatial
resolution of ecdysone transduction along the genome is on the order of
hundreds of base pairs. Additionally, this responsive gene architecture
suggests a role for Pol2-associated chromatin modifications. Further-
more, the widespread and early induction of genes encoding chromatin
remodeling factors like tou supports the idea that the secondary targets
may be determined in part by chromatin remodeling, and that cofac-
tors, in addition to TFs, play essential roles in specifying cellular
responses.

Our most significant observation is that the cell line-specific panel of
responsive genes can be predicted using the estimated titers of some TFs
together with their sequence specific binding motifs. In particular, br and
lola are strong predictors of restricted responses across all 41 cell lines.
We estimate that more than a hundred TFs are needed to achieve
maximal predictive accuracy, indicating that the interaction of many
TFs and cofactors convey divergent gene responses as previously re-
ported (Ihry and Bashirullah 2014).

We count ecdysone-responsive genes by looking for statistically
significant changes in expression. It is important – even if elemen-
tary – to appreciate that a survey of this kind cannot assess biological

significance. It seems likely that most large changes in expression play
important roles, but the majority of responses are small. A twofold
threshold is often selected, but is arbitrary. On the one hand, as we
have noted above, consistent subthreshold changes in members of
important protein complexes are likely to be important even if the
individual changes are subthreshold. We must also consider the possi-
bility that some ecdysone responses are incidental in the sense that the
receptor binds (directly or indirectly) to a promoter or enhancer se-
quence that evolved for other purposes. Or, alternatively, as is indicated
by the coresponsiveness of proximal genes transcribed on the same
strand, local chromatin modifications needed to activate a target gene
may have auxiliary effects in the genomic neighborhood. A response
that is physiologically unnecessary but not harmful will be maintained
if the components of the response are needed for other purposes, e.g.,
in other cell types. Whatever the biological significance (or lack of it)
for downstream cytological events of these minor expression changes,
understanding the regulatory events that cause them can be expected
to shed light on the regulatory syntax we are studying.

The catalog we have produced, along with our initial successes in
predicting ecdysone responses, indicate the potential of the system as a
model for hormone response. In our view these 41 cell lines, representing
41 individual systems, are governed by regulatory rules that are likely
identical, or nearly so, to those of in vivo Drosophila cells. They respond
to a common signal with diverse responses that must be specified by
differing initial states. More detailed studies of these responses, ex-
panded to include small RNAs and coupled with binding site maps
for relevant transcription factors, chromatin state profiling, and judi-
ciously designed perturbations, should make it possible to increase
the accuracy of prediction and elucidate the underlying biology of
hormone response in metazoans.

ACKNOWLEDGMENTS
We thank Yi Zou, Philip Knollman for discussions and help with the
microarray experiments, and Ram Podicheti and Doug Rusch for
initial data processing. J.B.B. was supported by National Human
Genome Research Institute (NHGRI) R00 HG006698 and Depart-
ment of Energy contract no. DE-AC02-05CH11231. S.E.C. was
funded by a contract from the NHGRI modENCODE Project contract
U01 HG004271 (Principal Investigator) and National Institutes of
Health (NIH) Grant R01 GM076655 under Department of Energy
contract no. DE-AC02-05CH11231. Work in Bloomington was
supported in part by the Indiana METACyt Initiative of Indiana
University, funded by an award from the Lilly Endowment, Inc. and NIH grant 2P40OD010949-10A1.

Author contributions: L.C. and P.C. conceived and designed the experiments. L.C. performed the experiments. M.S. and B.B. analyzed the data. P.C. contributed reagents, materials, and analysis tools. M.S. and P.C. wrote the paper. S.C. and B.B. assisted in revision of the manuscript.

LITERATURE CITED

Ables, E. T., and D. Drummond-Barbosa, 2010 The steroid hormone ecdysone functions with intrinsic chromatin remodeling factors to control female germline stem cells in Drosophila. Cell Stem Cell 7: 581–592.

Abraham, A., F. Pedregosa, M. Eickenberg, P. Gervais, A. Mueller et al., 2014 Machine learning for neuroimaging with scikit-learn. Front. Neuroinform. 8: 14.

Anders, S., and W. Huber, 2010 Differential expression analysis for sequence count data. Genome Biol. 11: R106.

Anders, S., A. Reyes, and W. Huber, 2012 Detecting differential usage of exons from RNA-seq data. Genome Res. 22: 2008–2017.

Anders, S., P. T. Pyl, and W. Huber, 2015 HTSeq—a Python framework to work with high-throughput sequencing data. Bioinformatics 31: 166–169.

Andres, A. J., and P. Cherbas, 1994 Tissue-specific ecdysone responses: regulation of the Drosophila genes Eip28/29 and Eip40 during larval development. Development 116: 865–876.

Andres, A. J., and P. Cherbas, 1994 Tissue-specific regulation by ecdysone: distinct patterns of Eip28/29 expression are controlled by different ecdysone response elements. Dev. Genet. 15: 320–331.

Ashburner, M., 1973 Sequential gene activation by ecdysone in polytene chromosomes of Drosophila melanogaster. I. Dependence upon ecdysone concentration. Dev. Biol. 35: 47–61.

Azoitei, A., and M. Spindler-Barth, 2009 DNA affects ligand binding of the ecdysone receptor of Drosophila melanogaster. Mol. Cell. Endocrinol. 303: 91–99.

Badenhorst, P. H., X. Xiao, L. Cherbas, S. Y. Kwon, M. Voas et al., 2005 The Drosophila nucleosome remodeling factor NURF is required for Ecdysteroid signaling and metamorphosis. Genes Dev. 19: 2540–2545.

Beckstead, R. B., G. Lam, and C. S. Thummel, 2005 The genomic response to 20-hydroxyecdysone at the onset of Drosophila metamorphosis. Genome Biol. 6: R99.

Bernardo, T. J., V. A. Dubrovskaya, H. Jannat, B. Maughan, and E. B. Dubrovsky, 2009 Hormonal regulation of the E75 gene in Drosophila: identifying functional regulatory elements through computational and biological analysis. J. Mol. Biol. 387: 794–808.

Bernhard, H. P., S. Lienhard, and U. Regenass, 1980 Isolation and characterization of mutator Drosophila cell lines, pp. 13–26 in Invertebrate Systems in Vitro, edited by Kurstak, E., K. Maramorosch, and A. Dubendorfer. Elsevier/North Holland Biomedical Press, Amsterdam.

Bodi, L., N. Zsindely, R. Gaspar, I. Kristo, O. Komonyi et al., 2012 Ecdysone induced gene expression is associated with acetylation of histone H3 lysine 23 in Drosophila melanogaster. PLoS One 7: e40565.

Braun, S. A., A. Azoitei, and M. Spindler-Barth, 2009 DNA-binding properties of Drosophila ecdysone receptor isoforms and their modification by the heterodimerization partner ultraspiracle. Arch. Insect Biochem. Physiol. 72: 172–191.

Breiman, L., 2001 Random forests. Mach. Learn. 45: 5–32.

Brown, J. B., N. Boley, R. Eisman, G. E. May, M. H. Stoiber et al., 2014 Diversity and dynamics of the Drosophila transcriptome. Nature 512: 393–399.

Cakouros, D., T. J. Daish, and S. Kumar, 2004 Ecdysone receptor directly binds the promoter of the Drosophila caspase dronc, regulating its expression in specific tissues. J. Cell Biol. 165: 631–640.

Carbonell, A., A. Mazo, F. Serras, and M. Corominas, 2013 Ash2 acts as an ecdysone receptor coactivator by stabilizing the histone methyltransferase Trr. Mol. Biol. Cell 24: 361–372.

Chanut-Delalande, H., Y. Hashimoto, A. Pelissier-Monier, R. Spokony, A. Dib et al., 2014 Pri peptides are mediators of ecdysone for the temporal control of development. Nat. Cell Biol. 16: 1035–1044.

Cherbas, L., K. Lee, and P. Cherbas, 1991 Identification of ecdysone response elements by analysis of the Drosophila Eip28/29 gene. Genes Dev. 5: 120–131.

Cherbas, L., X. Hu, I. Zhimulev, E. Belyaeva, and P. Cherbas, 2003 EcR isoforms in Drosophila: testing tissue-specific requirements by targeted blockade and rescue. Development 130: 271–284.

Cherbas, L., A. Willingham, D. Zhang, L. Yang, Y. Zou et al., 2011 The transcriptional diversity of 25 Drosophila cell lines. Genome Res. 21: 301–314.

Cherbas, P., L. Cherbas, S. S. Lee, and K. Nakaniishi, 1988 26-[125I]iodo- ponasterone A is a potent ecdysone and a sensitive radioligand for ecdysone receptors. Proc. Natl. Acad. Sci. USA 85: 2096–2100.

Chodankar, R. D., Y. Wu, B. J. Schiller, K. R. Yamamoto, and M. R. Stallcup, 2014 Hic-5 is a transcription coregulator that acts before and/or after glucocorticoid receptor genome occupancy in a gene-selective manner. Proc. Natl. Acad. Sci. USA 111: 4007–4012.

Currie, D. A., M. J. Milner, and C. W. Evans, 1988 The Growth and Differentiation In vitro of Leg and Wing Imaginal Disk Cells from Drosophila-Melanogaster. Development 102: 805–814.

D’Avino, P. P., S. Crispi, L. Cherbas, P. Cherbas, and M. Furia, 1995 The moulting hormone ecdysone is able to recognize target elements composed of direct repeats. Mol. Cell. Endocrinol. 113: 1–9.

Davis, M. B., I. SanGil, G. Berry, R. Olayokun, and L. H. Neves, 2011 Identification of common and cell type specific LXXLL motif EcR cofactors using a bioinformatics refined candidate RNAi screen in Droso phila melanogaster cell lines. BMC Dev. Biol. 11: 66.

Debec, A., 1978 Haploid cell cultures of Drosophila melanogaster. Nature 274: 255–256.

Depeuant, M. L., D. Fagegaltier, Y. Hu, K. Spirohn, A. Simcox et al., 2015 Discovery of progenitor cell signatures by time-series expression analysis during Drosophila embryonic cell immortalization. Proc. Natl. Acad. Sci. USA 112: 12974–12979.

Derrien, T., R. Johnson, G. Bussotti, A. Tanzer, S. Djebali et al., 2012 The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res. 22: 1775–1789.

Dressel, U., D. Thornberry, B. Altincicek, A. Paululat, M. Eggert et al., 1999 Alien, a highly conserved protein with characteristics of a corepressor for members of the nuclear hormone receptor superfamily. Mol. Cell. Biol. 19: 3383–3394.

Eads, B., A. Cash, K. Bogart, J. Costello, and J. Andrews, 2006 Troubleshooting microarray hybridizations. Methods Enzymol. 411: 34–49.

Echaller, G., and A. Ohannessian, 1969 Isolement, en culture in vitro, de lignees cellulaires diploides de Drosophila melanogaster. R. Acad. Sci. 268: 1771–1773.

Fallon, A. M., and A. Gerenday, 2010 Ecdysone and the cell cycle: investigations in a mosquito cell line. J. Insect Physiol. 56: 1396–1401.

Filippakopoulos, P., and S. Knapp, 2014 Targeting bromodomains: epigenetic readers of lysine acetylation. Nat. Rev. Drug Discov. 13: 337–356.

Garbuzov, A., and M. Tatar, 2010 Hormonal regulation of Drosophila microRNA let-7 and miR-125 that target innate immunity. Fly (Austin) 4: 306–311.

Gatell, E., L. Gissmann, R. Shrestha, N. Plus, H. Pfister et al., 1980 Characterization of two tumorous blood cell lines of Drosophila melanogaster and the viruses they contain, pp. 517–533 in Invertebrate Systems in Vitro, edited by Kurstak, E., K. Maramorosch, and A. Dubendorfer. Elsevier/North Holland Biomedical Press, Amsterdam.

Gauher, Z., L. V. Sun, S. Hua, C. E. Mason, F. Fuchs et al., 2009 Genomic mapping of binding regions for the Ecdysone receptor protein complex. Genome Res. 19: 1006–1013.

Gauthier, S. A., E. VanHaften, L. Cherbas, P. Cherbas, and R. S. Hewes, 2012 Cryptochrome, the Drosophila melanogaster ATF4, is a specific coactivator for ecdysone receptor isoform B2. PLoS Genet. 8: e1002883.
Gene Ontology Consortium, Blake, J. A., M. Dolan, H. Drabkin, D. P. Hill et al., 2013 Gene Ontology annotations and resources. Nucleic Acids Res. 41: D530–D535.

Gonsalves, S. E., S. J. Neal, A. S. Kehoe, and J. T. Westwood, 2011 Genome-wide examination of the transcriptional response to ecdsysteroids 20-hydroxyecdysone and ponasterone A in Drosophila melanogaster. BMC Genomics 12: 475.

Gupta, S., J. A. Stamatoyannopoulos, T. L. Bailey, and W. S. Noble, 2007 Quantifying similarity between motifs. Genome Biol. 8: R24.

Hammonds, A. S., C. A. Bristow, W. W. Fisher, R. Weiszmann, S. Wu et al., 2013 Spatial expression of transcription factors in Drosophila embryonic organ development. Genome Biol. 14: R140.

Heinz, S., C. Benner, N. Spann, E. Bertolino, Y. C. Lin et al., 2010 Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. Mol. Cell 38: 576–589.

Illy, R. I., and A. Bashirullah, 2014 Genetic control of specificity to steroid-triggered responses in Drosophila. Genetics 196: 767–780.

Justiniano, S. E., A. Mathew, S. Mitra, S. N. Manivannan, and A. Simcox, 2012 Loss of the tumor suppressor Pten promotes proliferation of Drosophila melanogaster cells in vitro and gives rise to continuous cell lines. PLoS One 7: e31417.

Karpova, N., Y. Bobinnev, S. Fouix, P. Huitorel, and A. Debec, 2006 Jupiter, a new Drosophila protein associated with microtubules. Cell Motil. Cytoskeleton 63: 301–312.

Kellner, W. A., E. Ramos, K. Van Bortle, N. Takenaka, and V. G. Corces, 2012 Genome-wide phosphoacetylation of histone H3 at Drosophila enhancers and promoters. Genome Res. 22: 1081–1088.

Kilpatrick, Z. E., D. Calouros, and S. Kumar, 2005 Ecdysone-mediated up-regulation of the effector caspase DRICE is required for hormone-dependent apoptosis in Drosophila cells. J. Biol. Chem. 280: 11981–11986.

Koelle, M. R., W. S. Talbot, W. A. Seagraves, M. T. Bender, P. Cherbas et al., 1991 The Drosophila EcR gene encodes an ecdysone receptor, a new member of the steroid receptor superfamily. Cell 67: 59–77.

Kugler, S. J., E. M. Gehring, V. Wallkamm, V. Kruger, and A. C. Nagel, 2011 The Putzig-NURF nucleosome remodeling complex is required for ecdysone receptor signaling and innate immunity in Drosophila melanogaster. Genetics 188: 127–139.

Lee, H., C. McManus, D. Y. Cho, M. Eaton, F. Renda et al., 2014 DNA copy number evolution in Drosophila cell lines. Genome Biol. 15: R70.

Leonardi, J., R. Fernandez-Valdivia, Y. D. Li, A. A. Simcox, and H. Jafar-Nejad, 2011 Multiple O-glucosylation sites on Notch function as a buffer against temperature-dependent loss of signaling. Development 138: 3569–3578.

Li, T. R., and K. P. White, 2003 Tissue-specific gene expression and ecdysone-regulated genomic networks in Drosophila. Dev. Cell 5: 59–72.

Mosna, G., and S. Dollini, 1972 Morphological and chromosomal characterization of three new continuous cell lines of Drosophila melanogaster. Chromosoma 38: 1–9.

Nakagawa, Y., and V. C. Henrich, 2009 Arthropod nuclear receptors and their role in molting. FEBS J. 276: 6128–6157.

Niki, Y., T. Yamaguchi, and A. P. Mahowald, 2006 Establishment of stable cell lines of Drosophila germ-line stem cells. Proc. Natl. Acad. Sci. USA 103: 16325–16330.

Savakis, C., G. Demetri, and P. Cherbas, 1980 Ecdysteroid-inducible polypeptides in a Drosophila cell line. Cell 22: 665–674.

Sawatsubashi, S., T. Murata, J. Lim, R. Fujiki, S. Ito et al., 2010 A histone chaperone, DEK, transcriptionally coactivates a nuclear receptor. Genes Dev. 24: 159–170.

Schneider, I., 1972 Cell lines derived from late embryonic stages of Drosophila melanogaster. J. Embryol. Exp. Morphol. 27: 353–365.

Sedkov, Y., E. Cho, S. Petruk, L. Cherbas, S. T. Smith et al., 2003 Methylation at lysine 4 of histone H3 in ecdysone-dependent development of Drosophila. Nature 426: 78–83.

Shlyueva, D., C. Stelzer, D. Gerlach, J. O. Yanez-Cuna, M. Rath et al., 2014 Hormone-responsive enhancer-activity maps reveal predictive motifs, indirect repression, and targeting of closed chromatin. Mol. Cell 54: 180–192.

Simcox, A., S. Mitra, S. Truesdell, L. Paul, T. Chen et al., 2008 Efficient genetic method for establishing Drosophila cell lines unlocks the potential to create lines of specific genotypes. PLoS Genet. 4: e1000142.

St Pierre, S. E., L. Ponting, R. Stefan cis, and P. McQuillon Flybase Consortium, 2014 FlyBase 102—advanced approaches to interrogating FlyBase. Nucleic Acids Res. 42: D780–D788.

Tominaga, M., E. Nishihara, T. Oogami, M. Iwasaki, Y. Takagi et al., 2010 Neurite elongation from Drosophila neural BG2-c6 cells stimulated by 20-hydroxyecdysone. Neurosci. Lett. 482: 250–254.

Tsay, C. C., H. Y. Kao, T. P. Yao, M. McKeown, and R. M. Evans, 1999 SMRTER, a Drosophila nuclear receptor coregulator, reveals that EcR-mediated repression is critical for development. Mol. Cell 4: 175–186.

U, K., R. Ueda, and T. Miyake, 1987 Cell lines from imaginal discs of Drosophila melanogaster. In Vitro Cell. Dev. Biol. 23: 707–711.

U, K., S. Nishihara, M. Sakuma, S. Togashi, R. Ueda et al., 1994 Newly established cell lines from Drosophila larval CNS express neural specific characteristics. In Vitro Cell. Dev. Biol. Anim. 30A: 209–216.

Vanolst, L., C. Fromental-Ramain, and P. Ramain, 2005 Toutatis, a TIPS-related protein, positively regulates Pannier function during Drosophila neural development. Development 132: 4327–4338.

Yao, T. P., B. M. Forman, Z. Jiang, L. Cherbas, J. D. Chen et al., 1993 Functional ecdysone receptor is the product of EcR and Ultra-spireacle genes. Nature 366: 476–479.

Zhu, J., L. Chen, G. Sun, and A. S. Raikhel, 2006 The competence factor beta Ftz-F1 potentiates ecdysone receptor activity via recruiting a p160/SRC coactivator. Mol. Cell. Biol. 26: 9402–9412.

Zraly, C. B., and A. D. Dingwall, 2012 The chromatin remodeling and mRNA splicing functions of the Brahma (SWI/SNF) complex are mediated by the SNR1/SNF5 regulatory subunit. Nucleic Acids Res. 40: 5975–5987.

Zraly, C. B., F. A. Middleton, and A. D. Dingwall, 2006 Hormone-response genes are direct in vivo regulatory targets of Brahma (SWI/SNF) complex function. J. Biol. Chem. 281: 35305–35315.

Communicating editor: M. Boutros