Gene expression profiling of brakeless mutant Drosophila embryos

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The transcriptional co-regulator Brakeless performs many important functions during Drosophila development, but few target genes have been identified. Here we use Affymetrix microarrays to identify Brakeless-regulated genes in 2–4 h old Drosophila embryos. Robust multi-array analysis (RMA) and statistical tests revealed 240 genes that changed their expression more than 1.5 fold. We find that up- and down-regulated genes fall into distinct gene ontology categories. In our associated study [2] we demonstrate that both up- and down-regulated genes can be direct Brakeless targets. Our results indicate that the co-repressor and co-activator activities of Brakeless may result in distinct biological responses. The microarray data complies with MIAME guidelines and is deposited in GEO under accession number GSE60048.

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S p e c i f i c a t i o n s T a b l e  [ p l e a s e f i l l i n r i g h t - h a n d c o l u m n o f t h e t a b l e b e l o w ]

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More specific subject area
Type of data
How data was acquired
Data format
Experimental factors
Experimental features
Data source location
Data accessibility

**Value of the data**

- This data significantly extends the number of Brakeless-target genes
- The data shows that more genes are down-regulated than up-regulated in brakeless mutant embryos
- Down-regulated and up-regulated Brakeless target genes fall into distinct gene ontology clusters

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Fig. 1. Brakeless-repressed and Brakeless-activated genes fall into distinct GO-categories. Grouping of genes into enrichment clusters revealed by DAVID functional annotation analysis. (A) Genes down-regulated in bks mutant embryos from the expression array and (B) up-regulated genes. The enrichment score depicts the geometric mean in log2-scale of the member’s P-values within a cluster.

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The data is deposited in GEO under accession number GSE60048. http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=olqdikeqjinutraf&acc=GSE60048
1. Data

To identify Brakeless target genes in the early embryo, RNA was isolated from embryos derived from brakeless (bks278) germline clones, which lack the maternal contribution of Brakeless. This was compared to RNA from germline clone embryos generated with the unmutagenized FRT chromosome on which the bks278 allele was induced [8]. The RNA was converted to cDNA and hybridized to an Affymetrix array. Mis-regulated genes that change their expression more than 1.5 fold were identified (supplementary material Table 1). We compared our gene list to an RNA-seq dataset that distinguishes maternal from zygotic transcripts in Drosophila embryos using polymorphisms [7]. The Brakeless-regulated genes were categorized as being maternally, zygotically, or maternally and zygotically (matzyg) derived (supplementary material Table 1). They were also subjected to functional annotation analysis using DAVID [3], which groups genes into clusters based on co-association with gene ontology (GO) terms (supplementary material Table 1). As shown in Fig. 1, up-regulated and down-regulated gene fall into distinct GO clusters.

2. Experimental design, materials and methods

2.1. Germline clones

The FLP–FRT dominant female sterile technique previously described in Ref. [1] was used to generate brakeless germline clones. The bks278 allele was used, which has a 345 bp deletion that causes a frame shift at aa 741 resulting in addition of 79 novel amino acids [5]. It was outcrossed with a w1118 strain to remove potential second-site mutations. FRT2G13 bks278/CyO females were crossed with males of the genotype hs-FLP/Y; FRT2G13 ovoD1/CyO, derived from FRT2G13 ovoD1/T(1;2)OR64/CyO (Bloomington stock #4344). Offspring larvae were heat-shocked for 3 h at 37 °C on days 3, 4 and 5 after egg-laying to induce expression of the FLP recombinase. Cy+ females were crossed to FRT2G13 bks278/CyO males and embryos were collected, dechorionated using bleach, and directly frozen in Trizol at −80°C for RNA isolation. Corresponding crosses, embryo collection and RNA isolation was performed with an unmutagenized FRT2G13 chromosome.

2.2. Microarray analysis

Staged 2–4 h old embryos were collected and immediately frozen at −80°C prior to RNA extraction. Total RNA was isolated using TRIzol (Invitrogen) and purified using an RNasea kit (Qiagen) according to the manufacturer’s protocols. Forty ul of embryos were used for each of three biological replicates of embryos derived from FRT2G13 bks278 or FRT2G13 c px sp control germline clones. cDNA probes were hybridized to an Affymetrix Drosophila gene chip (version 2). The intensity values were normalized and summarized with the robust multi-array analysis (RMA) method [6], using R (www.R-project.org) and the Bioconductor package [4].

2.3. Statistical analysis

After RMA normalization, 640 probes passed a > 1.5 fold difference in median expression levels between the conditions investigated. They were subjected to a two tailed unpaired Student’s t-test with a 95% confidence level, followed by Bonferroni’s correction for multiple tests, resulting in a P-value cut-off at 0.000078125. After removal of probe-sets targeting duplicates and pseudogenes, 240 genes remained.

2.4. GO analysis

The lists of genes with significantly changed expression levels containing 174 down- and 66 up-regulated identifiers were used as input lists for the DAVID Functional Annotation Clustering tool [3]. The tool provides analysis of annotation content and gene ontology term enrichments, to highlight the most relevant GO terms associated with a gene list. The enrichment score is a geometric mean of the member’s P-values in a -log scale within an annotation cluster.
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Appendix A. Supplementary material

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.dib.2015.08.033.

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