Transcriptional landscape of the Drosophila wing

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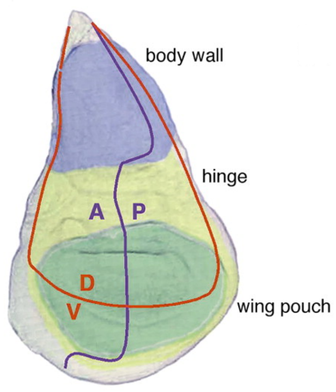
Abstract

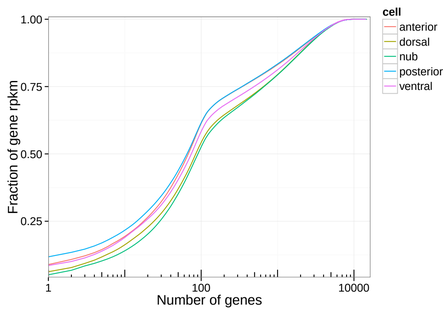
The fruitfly (*Drosophila melanogaster*) is one of the most studied model organisms, however only few transcriptomics and epigenetics studies by next-generation sequencing technologies are available for specific tissues along different developmental stages. In the current study we aim to profile RNAseq from five different wing compartments in three developmental stages, namely third instar larva, white pupa and late pupa. Preliminary RNA-seq data are available for the five sub-compartments of the wing imaginal discs in the third instar larva. We identified sets of commonly expressed genes as well as compartment-specific genes. A comparison of the splicing patterns shows that differences in splicing are negligible when compared to differences in the gene expression. The strand-specificity of the RNA-seq protocol allowed us to identify about 40 sense-antisense gene pairs with a significant level of correlation of expression. GO analysis of the coding genes with overlapping antisense non-coding transcript reveals an enrichment for development-related terms and encourages a possible regulation for at least some of the antisense genes.

Introduction

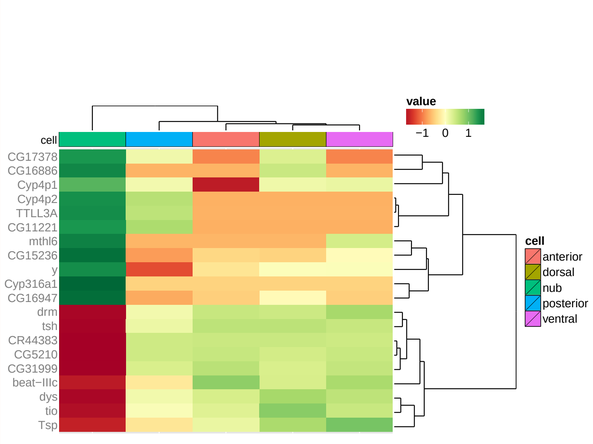
The third instar larva (L3) wing imaginal disc can be divided in 4 pairwise opposing compartments depending on the axial patterning. The cells in the pouch give rise to the adult wing. Cells from the 5 compartments were FACS sorted based on different marker genes.
Anterior (A): *engrailed*-GFP-; Posterior (P): *engrailed*-GFP+;
Dorsal (D): *apterous*-GFP+; Ventral (V): *apterous*-GFP-;
pouch: *nubbin*-GFP+;

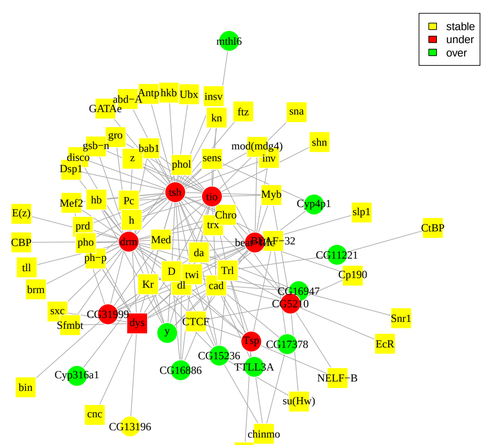


Gene expression



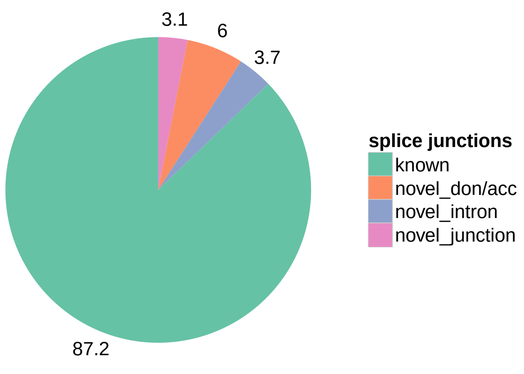
Only around 100 genes captures 60% of the transcriptional output in all compartments. They are mostly genes coding for ribosomal proteins. Their relevance in development is well established as mutants for these loci (*minute* mutants) have a slower and impaired development[1].

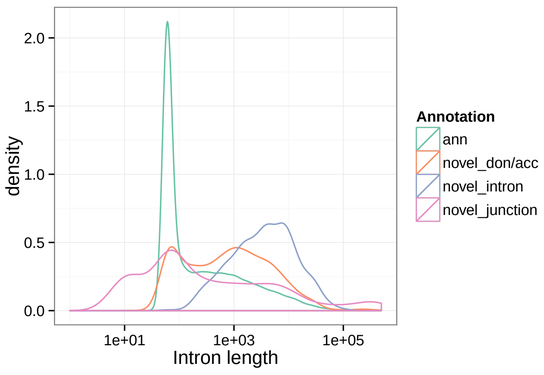




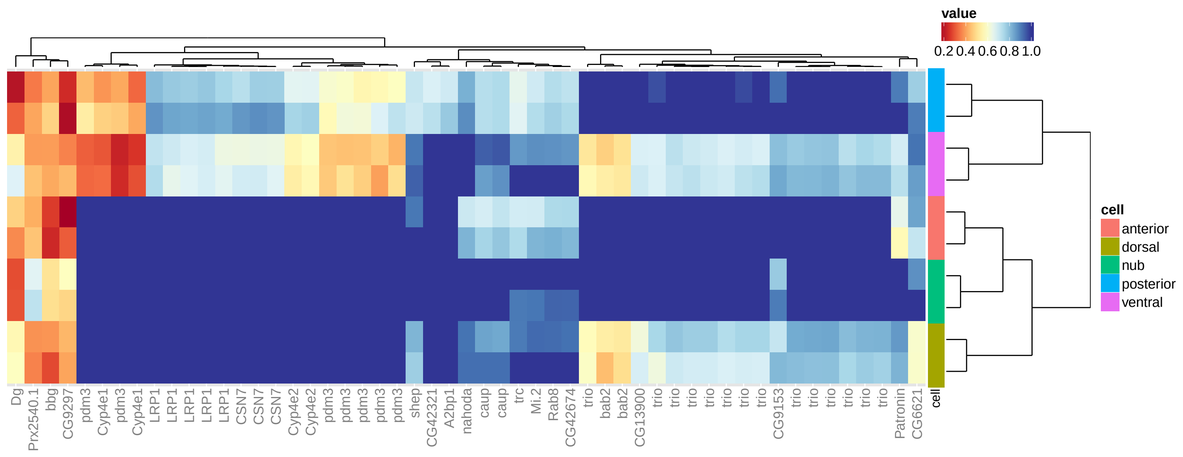
Strikingly, most of the differentially regulated genes in the wing pouch are known to act as transcription factors and/or belong to networks of transcriptional regulators and chromatin modifiers. This may reflect a particular differentiation status of the cells of this domain respect to the other compartments.

Splicing





We find about 5,000 novel splice junctions, cumulatvely 17% of all detected ones (1,215 novel junctions: both splice sites unannoteted, 2,378 novel don/acc: only one splice site annotated, 1,458 novel intron: both splice sites annotated, but not the intron between them). The overlapping peak among all intron length distributions suggests that some of the novel junctions may be valid.



Exon inclusion level (PSI) of the most variable exons (stdev¿=0.1 across cell types and stdev¡=0.1 within cell types). Most exons belong to the same gene and are annotated as constitutive. Few of them are annotated as alternative (e.g. Dg) and deserve further investigation.

Antisense transcription

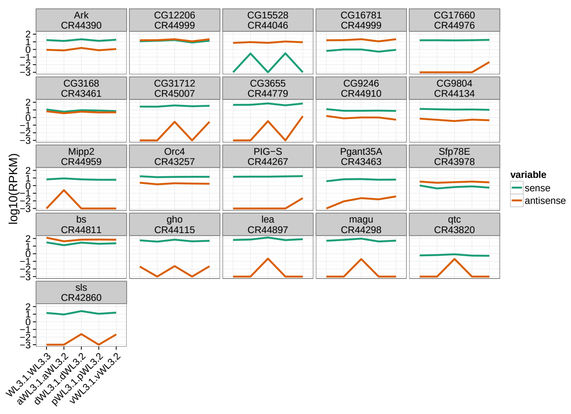
Out of 1477 annotated lncRNAs (FlyBase v5.54), 535 are antisense to protein coding genes.

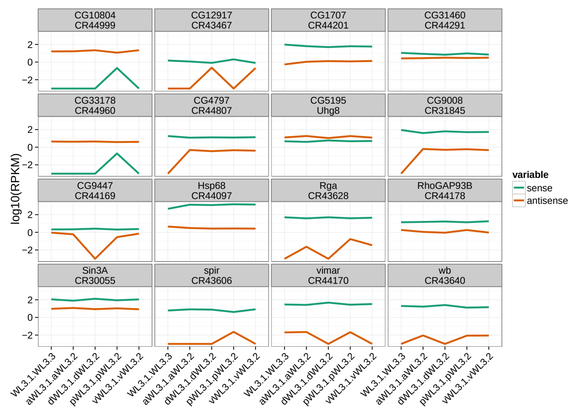
nbPairs	orientation
62	3tail-to-tail
101	5head-to-head
69	external
303	internal

Pvalue	Term
4.9e-12	single-multicellular organism process
2.4e-11	post-embryonic organ development
2.8e-11	imaginal disc morphogenesis
5.0e-11	organ morphogenesis
1.5e-10	post-embryonic morphogenesis
1.5e-10	instar larval or pupal development
6.0e-10	appendage morphogenesis
6.5e-10	imaginal disc-derived appendage development
3.4e-09	imaginal disc-derived wing morphogenesis
5.4e-09	wing disc development

Even though it is still debated whether antisense transcription is noise or functional[2], the GO term enrichment for development-related terms may suggest some functional implication in development.

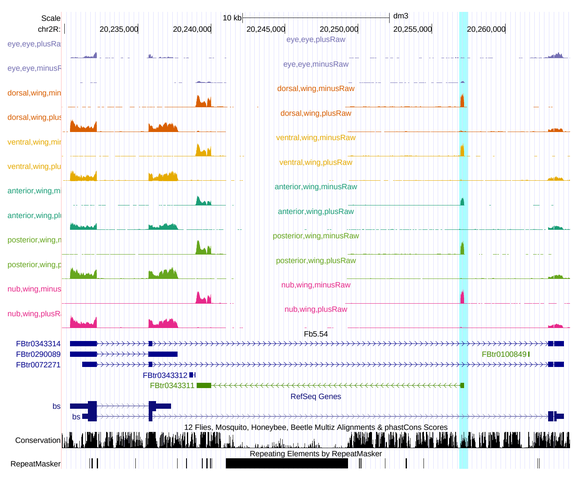
Correlation of sense-antisense pairs

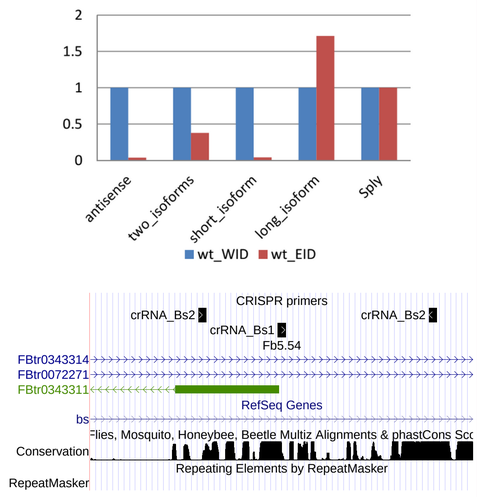




We find 21 and 16 positively and negatively correlated sense-antisense pairs, respectively (|cc|>0.8). Although the antisense gene is often lowly expressed, there are some documented regulatory mechanisms in mammals[3].

Among the correlated pairs, we initially selected *bs* for its role in wing vein formation[4].





bs antisense expression is negatively correlated with the long isoform of *bs*, which is even more evident when comparing wing and eye samples, and validated by qPCR. To validate a possible regulatory role of *bs* antisense on the isoform usage of the sense, we will use the CRISPR strategy with two approaches: 1) cut the region just upstream of the antisense TSS, 2) delete a 500 bp region surrounding the antisense TSS.

References

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