Drosophila melanogaster –
a versatile model in biology and medicine
The life cycle of *Drosophila melanogaster*
Large number of progeny
Drosophila culture tubes
Drosophila culture
Fly incubators, 18°C, 25°C, %70 humidity, 12:12 day:night cycle
The fly room
The fly room
<table>
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<th>Gene Index</th>
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<td>and mesoderm)</td>
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<td>Morphogenesis and organogenesis: index of genes active in the formation of various organs (including eyes, gut, heart,</td>
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<td>Imaginal Discs: The Genetic and Cellular Logic of Pattern Formation, by Lewis Held Jr.</td>
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FlyBase

A Database of Drosophila Genes & Genomes

QuickSearch

Simple  Expression  Phenotype  GO  References  Human Disease  Data Class

Species:  include non-Dmel species
Enter text:

Note: Wild cards (*) can be added to your search term

Commentary

See all commentaries

New custom configurations of RNA-Seq profiles in GBrowse

Nov 10, 2014. FlyBase introduces new user-configurable display options for RNA-Seq profiles in GBrowse as part of the FB2014_06 update. By clicking on the small "wrench" icon in the track title bar a pop-up configuration menu can be accessed that allows users to customize several aspects of the display. (More)
Why *Drosophila*?

fly genome has been completely sequenced and annotated

Encodes 14,000 genes on four chromosomes

nearly 75% of disease-related genes in humans have functional orthologs in the fly

Overall identity

at nucleotide level or protein sequence between fly and mammal is usually approximately **40% between homologs**;
in conserved functional domains, it can be 80 to 90% or higher
Why *Drosophila*?

- The fly may be considered multiple model organisms, each with its own specific advantages, defined by developmental stage:
  - the embryo,
  - the larva,
  - the pupa,
  - and the adult
The embryo is often used in fundamental developmental studies examining
- pattern formation
- cell fate determination,
- organogenesis,
- and neuronal development and axon pathfinding.
The larva, particularly the wandering third instar larva, is routinely used to *study developmental and physiological processes* as well as some *simple behaviors such as foraging*. 

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**The Larva**

The larva, particularly the wandering third instar larva, is routinely used to **study developmental and physiological processes** as well as some **simple behaviors such as foraging**.
The future adult structures of the fly are contained within the larva as imaginal discs.
The study of the molecular and genetic mechanisms underlying imaginal disc developmental processes in the **pupa** has provided significant insight not only to fly biology but also to human biology.

The pupa is an appropriate model to investigate certain developmental processes.
The adult fly is a very sophisticated and complex organism not unlike higher organisms.
Organs

digestive tract
nervous system
body organisation
circulation excretion skeleton muscles
digestive tract
body organisation
nervous system
circulation excretion skeleton muscles

A. Prokop
Body organisation

- head
- thorax
- abdomen

A. Prokop
Moving

- bone
- tendon
- muscle
- bone cell (osteoblast)
- bone-tendon junction (enthesis)
- tendon
- muscle-tendon junction
- muscle
- tendon fibroblast
- collagen fibres

see close-up

- muscle
- cuticle
- tonofilament
- epidermis (skin cell)
- cytoskeletal array
- tendon matrix
- cuticle

A. Prokop
Circulation and blood
Excretion

- Kidney
  - Glomerulus
  - Tubule

- Blood
- Artery
- Vein

- Ureter (Urine)

- Thoracic (Garland) Nephrocyte
- Abdominal (Pericardial) Nephrocyte

Malpighian tubules

A. Prokop
The Hox complex
**Human**

**Mouse**

**Zebrafish**

**Drosophila**

### WT

**PAX6^+/−**
- Cornea opaque
- Iris absent
- Retina degenerate
- Lens opaque
- Aqueous humor of eyeball increased pressure

**Pax6^−/−**
- Eye decreased size
- Lens fused to cornea
- Iris morphology absent
- Anterior chamber absent

**pax6b^−/−**
- Eye decreased size
- Lens decreased size
- Retina malformed

**ey^−/−**
- Eye absent
the fly genome contains two types of genes:

those with sequence homology with human genes and those that have no obvious human homologs.
Genetic strategies to tackle neurological diseases in fruit flies

experimental strategies that build upon PhiC31 recombination-mediated cassette exchange (RMCE) to insert DNA

CRISPR/Cas9 to edit genomes

P[acman]/FlyFos genomic libraries to rescue mutants and determine protein localization

Minos-mediated integration cassette (MiMIC) to mutate genes and tag proteins

GAL4/UAS approaches to express transgenes
Reverse and forward genetic approaches

(a) Reverse genetics
- Identification of fly homologue(s)
- Manipulation of fly gene
  - Available mutations (EMS, transposons, deletions) or RNAis
  - Targeted gene disruption (CRISPR/Cas9)
  - Overexpression of fly/human gene (cDNAs or genomic rescue constructs)
- Detailed phenotypic characterization
- Modifier screens
- Better understanding of disease and potential therapeutic research

(b) Forward genetics
- Isolation of mutants displaying neurological defects
- Unbiased genetic screen (EMS or transposon-mediated mutagenesis, deletion library or RNAi screen)
- Mapping the causative mutation (For EMS and deletion screens)
- Detailed phenotypic characterization & Modifier screens
- Assessing if human homolog is associated with disease
Methods to model *Drosophila* for assessing disease variants

(a) Binary expression systems

<table>
<thead>
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<th>Expression System</th>
<th>Transcriptional Activator (TA)</th>
<th>Effector</th>
<th>Suppressor of TA</th>
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<td>LexA/LexAop</td>
<td>LexA</td>
<td>LexAop</td>
<td></td>
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(b) Expression of WT or disease-associated variant cDNAs

- Severe LOF or null
  - Gene of interest
  - Phenotypic analyses
    - Effector: WT human/fly cDNA
    - Effector: Human/fly cDNA w/variant

*Ubiquitous or tissue-specific promoter
  e.g. Actin, n-Syb, GMR

Phenotypic analyses and assessing rescue
The Gal4 UAS System

signals that activate normal expression of gene H

Gal4 gene

regulatory sequence of gene H
downstream of Gal4

Gal4 protein

gene G

Uas element

downstream of gene G

protein G
Introducing disease-associated variants in fly locus

(c') Introducing conserved variants via genomic rescue constructs

(c'') Introducing variants in fly gene by homologous recombination (HR)

(c''') Introducing variants by CRISPR based genome editing

- Severe LOF or null
- Genomic rescue construct
- WT fly gene
- Fly gene with variant
- WT human gene
- Human gene with variant
- Phenotypic analyses and assessing rescue

* Disease-associated variant

* Modified fly residue relevant to the human variant
Generation of protein traps and T2A-Gal4 strategy using MiMIC elements

(a) Generation of protein traps and T2A-GAL4 strategy using MiMIC elements

Gene locus Available MiMIC line or CRISPR/Cas9 mediated MiMIC insertion

(a’) Gene trap

MiL attP polyA yellow+ attP MiR

RMCE

(a’’) Protein trap

attB

OR

attB

RMCE

(a’’) T2A-GAL4 insertion

Transcription

Partial mRNA

Translation

Gene trap + GAL4

Truncated protein

T2A

GAL4

- Protein expression pattern
- deGradFP strategies

Fly cross

- Gene/protein expression pattern
- Assessing/comparing rescue ability of cDNAs upon loss of the gene

UAS

Fluorescent protein

UAS

WT human-fly cDNA

UAS

Human/fly cDNA w/variant
Conditional gene inactivation

INDUCING SIGNAL ACTIVATES PROMOTER OF Flp GENE

FLP CATALYSES MITOTIC CROSSING-OVER AND RECOMBINATION

cell of fly heterozygous for mutant gene X and homozygous for Frt (FLP recombinase target)

maternal chromosome

paternal chromosome

mutant gene X

Frt

CHROMOSOME REPLICATION

FLP recombinase

mRNA

INdUCING SIGNAL ACTIVATES PROMOTER OF Flp GENE

FIP gene

cell homozygous for mutant gene X

cell homozygous for normal gene X

clonal proliferation gives homozygous patches in wing

CELL DIVIDES
Conditional gene inactivation

(b) Conditional gene inactivation using MiMIC and flippase-dependent cassette inversion

(b’) Flip-Flop cassette

(b”) FlpStop cassette

Wild type (WT) tissue with GFP-tagged protein

Protein trap (PT) orientation

Gene trap (GT) orientation

Non-disrupting (ND) orientation

Disrupting (D) orientation

Mutant (mut) tissue expressing mCherry

WT tissue

Mutant tissue

Conditional gene disruption

Rescue
The homology between fly and human genes can vary widely and many fly genes have more than one human homolog.

Fly genome contains approximately 16,000 genes
- ~13,000 encode proteins
- More than 60% have human homologs

Among the ~8000 fly genes with human homologs, ~3500 have multiple human homologs
- ~4500 have only one

(Eyre et al. 2007; HCOP 2014).
Simplified evolutionary patterns of essential genes and their association with Mendelian disorders
a number of fly genes that are not essential for viability are also linked to Mendelian diseases:

null alleles of homologs of genes that cause

- familial forms of Alzheimer’s disease \(^{(\text{App1; Luo et al. 1992})}\),
- Parkinson’s disease \(^{(\text{parkin; Pesah et al. 2004 and Lrrk; Lee et al. 2007})}\),
- Duchenne muscular dystrophy \(^{(\text{Dystrophin; Christoforou et al. 2008})}\),
- Torsion dystonia \(^{(dTorsin; Wakabayashi-Ito et al. 2011})\),
- Usher syndrome \(^{(\text{CAD99C; D’Alterio et al. 2005 and Sans; Demontis and Dahmann 2009})}\), and
- Zellweger syndrome \(^{(\text{pex2, pex10, and pex16; Chen et al. 2010; Nakayama et al. 2011})}\)
Accelerating functional annotation of *Drosophila* and human genomes
Genetic strategies used in *Drosophila*

**Reverse genetics**
- Human disease-causing gene
  - Identify the fly homolog
    - Lower or abolish expression of the fly homolog
      - Targeted gene disruption (CRISPR)
      - Transposon-mediated mutagenesis
      - Gene silencing (RNAi, CRISPR)
    - Or overexpression of fly or human gene
  - Phenotype analysis
    - Insight into function of known disease gene

**Forward genetics**
- Unbiased screening of mutations or reduced gene activity for a specific phenotype
  - Chemical mutagenesis (EMS)
  - Transposon-mediated mutagenesis
  - Gene silencing (RNAi)
  - Deficiency library
- Phenotypic analysis
  - Map the causative gene (EMS and deficiency library)
  - Gene analysis
    - Identify the human homolog
    - Reveal new disease gene

**Diagnostic strategy**
- Whole exome sequencing (WES) of patient
  - New disease-causing gene candidate
    - Identify the fly homolog
      - Engineer a loss-of-function (LOF) fly by inserting the GAL4 cassette in the endogenous locus (LOF-GAL4 fly). GAL4 expression is controlled by the endogenous promoter
    - Phenotypic analysis of the LOF-GAL4 fly
      - Create a transgene of human WT UAS-cDNA
    - Express human WT UAS-cDNA in the LOF-GAL4 fly. Test for rescue of the phenotypes
      - Gene analysis
        - Identify disease-causing gene
        - Map rare disease-associated variants
Nervous system assays

The nervous system in adult flies

Electroretinogram (ERG)

An ERG provides a recording of light-induced photoreceptor activity. The ERG amplitude (green bracket) indicates the depolarization of photoreceptors, whereas the on/off transient spikes (black arrows) correspond to changes in postsynaptic potential.

Control

Young

Old

Recording electrode

Reference electrode

Marf is required for mitochondrial fusion and quality control. Loss of Marf leads to an age-dependent decrease in the ERG amplitude and loss of the on/off transients, indicating photoreceptor neurodegeneration (our unpublished observations).

Giant fiber system recordings

The giant fiber (GF) recording is the electrophysiological output of the escape response in fly. The recording is performed by stimulating the giant fiber through the eye (e.g., using electrodes; 8 stimulations are shown below) and recording the depolarization of the tergotrochanteral muscles (jump muscles, or TTMs) and the dorsal longitudinal muscles (flight muscles, or DLMs).

SOD1 overexpression

mV

Time

Wing injury assay

A simple cut in the anterior wing margin induces axonal degeneration of the peripheral nerve. Several different kinds of injury-induced axonal phenotypes can be observed, including broad axonal loss, local axonal loss, thick axon endings and misrouting of axonal projections.

Control

highwire mutant

The distal portions of the axons degenerate (arrow)

In the absence of highwire, an E3 ubiquitin ligase, axon degeneration is suppressed

Synaptotagmin

Control

mV

Time

Partial loss of Synaptotagmin, a synaptic vesicle Ca²⁺ sensor, causes reduced exocytosis. This is detected as a smaller excitatory junction potential amplitude.

The nervous system in Drosophila larvae

Neuromuscular junction electrophysiology

The larval neuromuscular junction (NMJ) is a model synapse to study neurotransmission. An electrophysiological NMJ recording is performed by stimulating the motor neuron axons and recording the depolarization change in the larval body wall muscles. Mutants exhibit altered excitatory junction potential amplitude, suggesting a neurotransmission defect.

Dissected larval preparation

Ventral nerve cord

Brain

Body wall muscle

Suction electrode

Stimulation via electrode

Measure voltage

Overexpression of SOD1, which metabolizes superoxide radicals, results in impaired neuronal function. The DLM response to repeated stimulation of the giant fiber is altered.
Cardiovascular assays

The circulatory system in adult flies

Heartbeat measurement

The heart rate can be measured in adult flies or dissected larvae by recording a video of the semi-intact beating heart. A movement detection algorithm, SOHA, can be used to produce M-mode images (shown below) to measure systole, diastole and rhythmicity.

Control

CryAB<sup>R120G</sup> OE

Dilation

Time

Dominant mutations in CryAB, a heat shock protein, cause dilated cardiomyopathy in humans. Similarly, overexpression of the mutated transgene CryAB<sup>R120G</sup> in flies results in a dilated dorsal vessel. Red arrow, diastolic diameter; yellow arrow, systolic diameter.

Image of M-mode trace adapted from Xie et al. (2013)

The circulatory system in Drosophila larvae

Field potential recordings in the heart

Electrophysiological recordings of heart field potentials can be made in both adult and larval hearts. In dissected larvae, the field potential is recorded using a fine glass electrode that touches the semi-intact heart.

Optical coherence tomography (OCT)

OCT is a in vivo non-invasive technique that uses light waves to generate a 3D image of biological tissues. Reconstructed images of the fly heart can be used to produce M-mode traces and assess internal cardiac chamber parameters.

Light is reflected onto awake flies

Backscattered light is detected and analyzed

Reconstruction of high-resolution subsurface images

Ca<sup>2+</sup> measurement

Cardiomyocytes require high levels of Ca<sup>2+</sup> to contract. Defects in Ca<sup>2+</sup> homeostasis are associated with numerous heart conditions, such as arrhythmia and heart failure. To assay cardiac Ca<sup>2+</sup> in flies, transgenic flies with cardiac-specific genetically encoded Ca<sup>2+</sup> indicators (GECIs) are used. The heart is dissected and imaged with a fast fluorescence camera, and changes in GFP fluorescence intensity are measured.

Recordings from adult hearts show positive deflections (depolarizing potential, black arrows) followed immediately by negative deflections (repolarizing potential, red arrows) that correlate with heart contraction and relaxation, respectively.

hdp<sup>2</sup> flies have a mutation in troponin, resulting in impaired Ca<sup>2+</sup> reuptake. The Ca<sup>2+</sup> peak intensity is increased in these flies.

KCNQ1 encodes a voltage-gated K<sup>+</sup> channel that is partially responsible for the rhythmic contraction of the heart muscle. In mutant hearts, the negative deflections do not immediately follow the positive depolarizing potentials, suggesting that repolarization is impaired.
Oenocyte and fat body assays

In flies, the fat body and oenocytes form the functional homologs of the liver.

Under fasting conditions, lipids in the form of FFAs are taken up from adipose tissue by the liver, and, in Drosophila, in the form of TAG from the fat body by specialized cells named oenocytes.

Humans
- Adipose tissue
- Liver
- Triglyceride (TAG)

Drosophila
- Fat body
- Oenocytes
- High lipid

Fat body and oenocyte function can be assessed by looking at the amount of lipids stored under different nutritional conditions. Two such assays are: lipid staining and coherent anti-Stokes Raman scattering (CARS) microscopy.

**Lipid-staining**

Oil Red-O is the most commonly used stain for lipids. Both adult and larval fat body and oenocytes can be dissected and stained. Under fed conditions, oenocytes do not accumulate fat, but mobilize lipids from the fat body under starvation conditions.

**Control**
- Fed: Fat body, Oenocytes
- Starved: Fat body, Oenocytes

**Hnf4 mutant**
- Fed: Fat body, Oenocytes
- Starved: Fat body, Oenocytes

**CARS microscopy**

CARS is an in vivo method that is able to give more accurate results than lipid staining. Larvae in different conditions are placed under the microscope and the lipid intensity, depicted here as an increase in intensity of yellow, is measured.

**Control**
- Fed: Fat body, Oenocytes
- Starved: Fat body, Oenocytes

**Lsd2 overexpression**
- Fed: Fat body, Oenocytes
- Starved: Lipid

Lsd2 regulates lipid homeostasis. Its overexpression results in excessive lipid storage and an overall increase in triglycerides. These flies don’t accumulate lipids in oenocytes when starved.

*Hnf4*-null flies are unable to mobilize stored lipids from the fat body, so they are sensitive to starvation.
Nephrocytes and Malpighian tubule assays

Malpighian tubules perform a range of kidney-associated functions, and several ion channels and transporters are shared between flies and mammals. A quantitative assay for transparency (not shown) can be used to assess Malpighian tubule function, enabling human diseases such as nephrolithiasis to be modeled.

Mammalian glomerular podocytes

The glomerular podocyte is an epithelial cell that plays an important role in the filtration of blood to produce urine. Glomerular podocytes create a filtration barrier (the slit diaphragm), through which blood is filtered.

The Drosophila analog of podocytes: nephrocytes

*Drosophila* nephrocytes, which share similarities to mammalian glomerular podocytes, filter hemolymph (blood) through nephrocyte diaphragms.

Nephrocyte filtration assay

The nephrocyte filtration assay is performed by expressing secreted GFP (ANF-GFP) from muscles. The GFP will then accumulate in nephrocytes.

**Control**
Pericardial nephrocyte

**sns knockdown**

Knockdown of *sns*, a transmembrane immunoglobulin-domain superfamily protein, leads to disrupted nephrocyte diaphragms. These mutants fail to accumulate GFP.