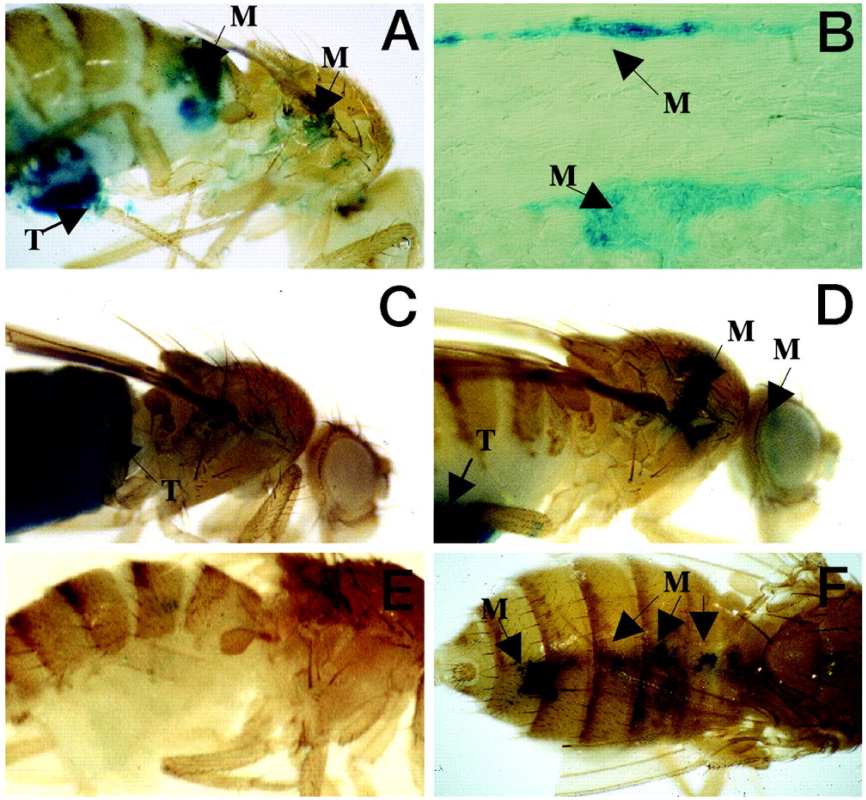
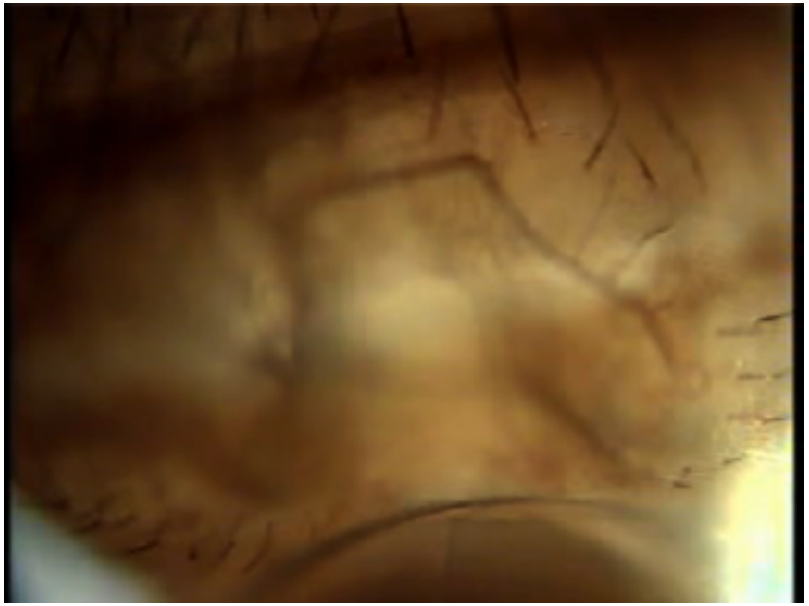
Fly genetics and screens for human disease Study





Yongkyu Park
2/2/15

Drosophila is a powerful model organism for the analysis of human disease genes

1) 74% of 1682 human disease genes have homologs in *Drosophila*.

2) A third of these genes (~ 500) are functionally equivalent between flies and humans.

3) Neurological disorders, developmental defects, metabolic/storage disorders, cancer, cardiovascular disease, the visual, auditory, and immune systems disorders.

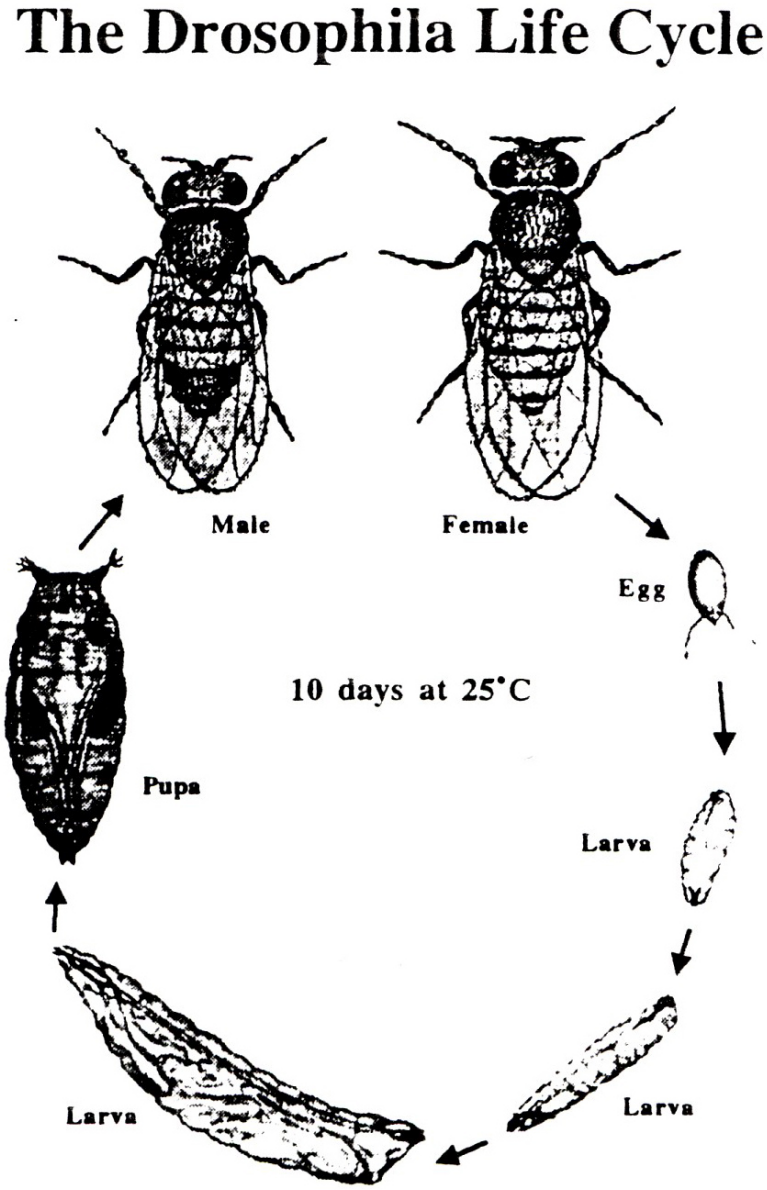
Drosophila Genetics

Thomas Hunt Morgan

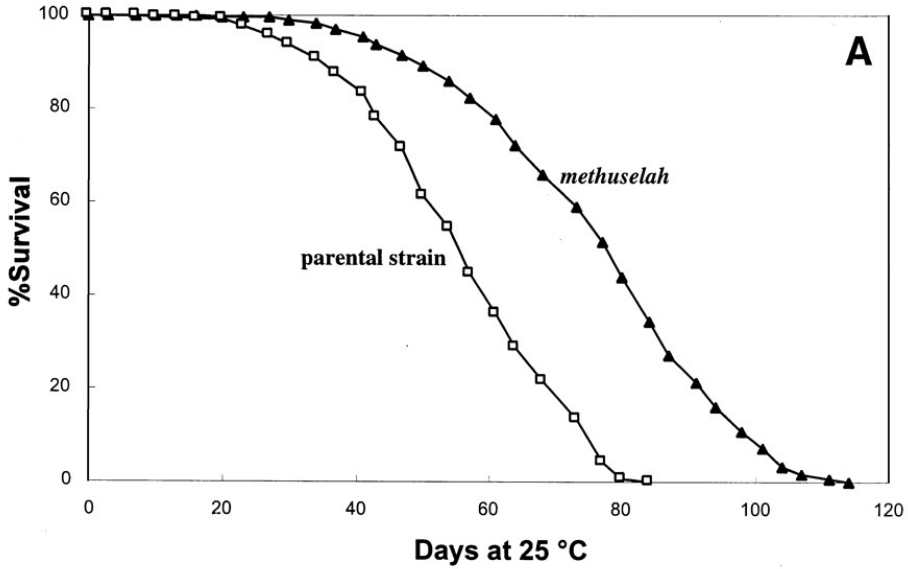
Why *Drosophila?*

1) Short life cycle
2) Large number of progeny
3) Ease of maintenance
4) Availability of stocks containing altered genes
5) Full sequences of ~ 12 *Drosophila* species
6) Model for development of multicellular organisms
7) Powerful molecular genetic techniques
8) Small genome (4 chromosome pairs)

1) Short life cycle, 2) Large number of progeny



Median lifespan: 40 ~ 50 days at 25°C



Lin et al., Science 1998

1 female : ~ 150 progenies

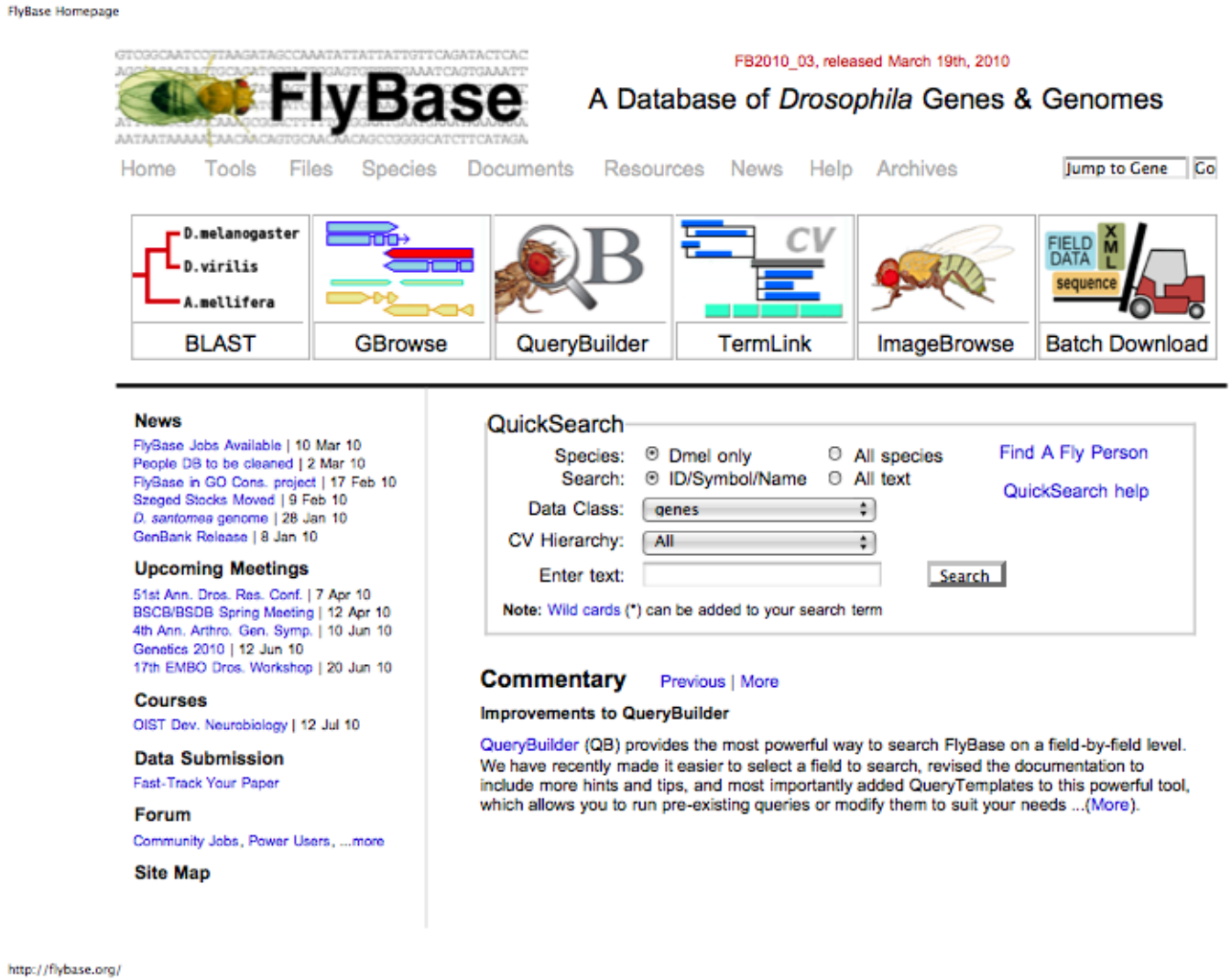
3) Ease of maintenance

Drosophila Culture

1. Media - agar, cornmeal, yeast extract, molasses
2. Vessel - vial, bottle
3. Microscope - 7X to 30X
4. Temperature - 18°C ~ 29°C
5. Virgin Collection - 6 ~ 8 hrs after eclosion
6. Mite Prevention - Cleaning

4) Availability of stocks containing altered genes

http://flybase.org



4) Availability of stocks containing altered genes

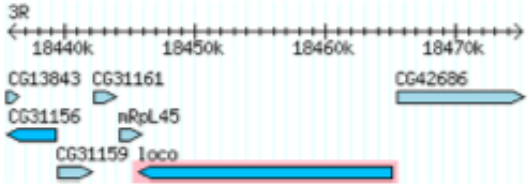
FlyBase

FB2010_03, released March 19th, 2010

Gene Dmel\loco

Home Tools Files Species Documents Resources News Help Archives Jump to Gene Go

Profile Manager Help Open All Close All

General Information			
Symbol	Dmel\loco	**Species**	*D. melanogaster*
Name	locomotion defects	**Annotation symbol**	CG5248
Feature type	protein_coding_gene	**FlyBase ID**	FBgn0020278
Gene Model Status	Current	**Stock availability**	10 publicly available
Genomic Location			
Chromosome (arm)	3R	**Recombination map**	
Cytogenetic map	94B6-94B8	**Sequence location**	3R:18,445,690..18,465,082 [-]
Genomic Maps **FlyBase GBrowse** **modENCODE GBrowse**		Decorated FastA Get genome region Gene region Get FastA	

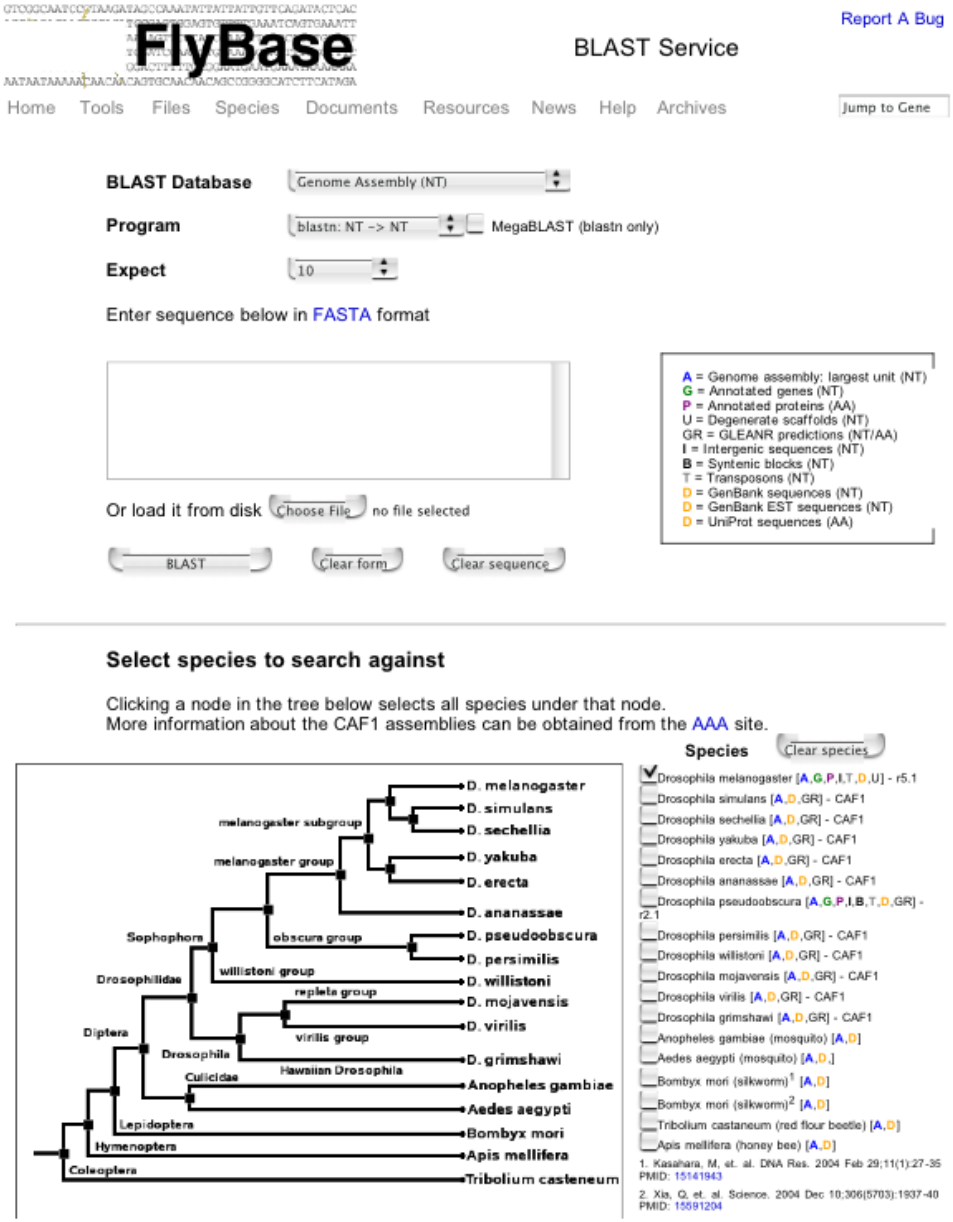
Summary Information

Automatically generated summary

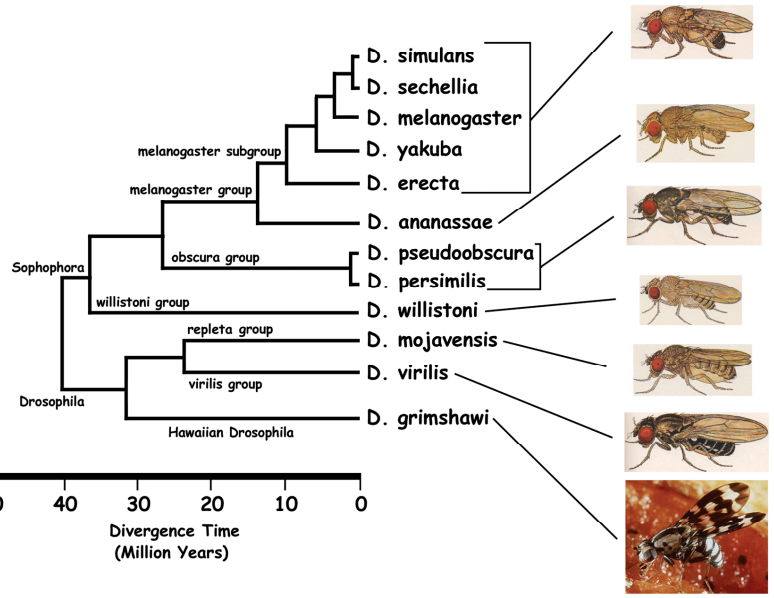
See sections below for more information

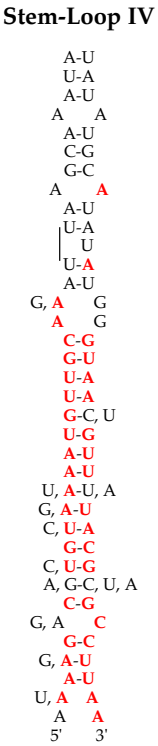
The gene ***locomotion defects*** is referred to in FlyBase by the symbol ***Dmel\loco*** (CG5248, FBgn0020278). It is a protein_coding_gene from *Drosophila melanogaster*. Its sequence location is **3R:18445690..18465082**. It has the cytological map location **94B6-94B8**. There is experimental evidence that it has the **molecular function**: G-protein alpha-subunit binding; GTPase activator activity. There is experimental evidence that it is involved in the **biological process**: regulation of G-protein coupled receptor protein signaling pathway; glial cell differentiation; asymmetric neuroblast division; asymmetric protein localization; dorsal/ventral axis specification, ovarian follicular epithelium; establishment of glial blood-brain barrier; cytoplasmic transport, nurse cell to oocyte; cortical actin cytoskeleton organization; septate junction assembly; ventral cord development. **34 alleles are reported**. The **phenotypes** of

5) Full sequences of ~ 12 *Drosophila* species



5) Full sequences of ~ 12 *Drosophila* species



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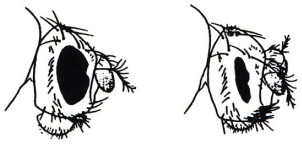
Si   CAAACUGAAGUCUUAAAAGACGUGUAAAAUGUUGCAAA----------------------------------------------------------------------------UUAAGCAAAUAUAUA-UGCAUAUAUGGGUAAUGUUUUACGCGCCUUAACCAGUCAAAA
Me   CAAACUGAAGUCUUAAAAGACGUGUAAAAUGUUGCAAA----------------------------------------------------------------------------UUAAGCAAAUAUAUA-UGCAUAUAUGGGUAACGUUUUACGCGCCUUAACCAGUCAAAA
Ya   AGAACUAAAGUCUUAAAAGACGUGUAAAAUGUUGCAAA----------------------------------------------------------------------------UUAAGCAAAUAUAUA-UGCAUAUAUGGGUAACGUUUUACGCGCCUUAACCAGUCAAAA
Er   AAAACUAAAGUCUUAAAAGACGUGUAAAAUGUUGCAAA----------------------------------------------------------------------------UUAAGCAAAUAUAUA-UGCAUACAUGGGUAACGUUUUACGCGCCUUAACCAGUCAAAA
An   AAAUAUAAAGUCUUGAAAGGCAUGCAAAAUGUUGCAAA----------------------------------------------------------------------------A-AUAUAUACAUAUAGUAUAUAAAAUGGUAAUGUUUUACGCGCCUUAACCAGCAACCA
Ps   UGGGCAAAAGUC-AAAAAGGCACGUAAAAUGUUGCAAA----------------------------------------------------------------------------A-----AAAUGCAU--UGCA---AUG-GUAACGUUUUACGAGCCUUAAAAAAUCUCAA
Wi   CAUUAAGACCUAUAAAAAGGCAUGUGAAAUGUUGCAAA----------------------------------------------------------------------------AAAAAAAAAAAAAGGGUAACGUUUUACGCGCCUUAACAAAUCCAUC
Mo   ACAAAAAUUAUUUUUAAAGGCAUGUAAAAUGUUGCAACAAAAAAAAAAAAAAUAAAAAAAAAUACAAAUUGUAAAUAAAUAAAAACGUAACGUUUUACGAGCCGAGA-AAAUAAAUGUAAAAUGUAGGAAAUUGUAACGUUAUACGCGCCUUAACAAUGAAAAA
Vi   AAACCAACUAUUU-UUAGGGCAUGUAUAAUGUUGCAGCACGCAUAA-------CAAAUAGCAAAUGUUAUACGUGCCGAGAAAAAAAGUGUUAAAAAAAAUAUAU-AUAUAGGAGUUAG--GAAAAGAAAUGUAACGUUAUACGUGCCUUAACAA-AUACUU
Con  ----------U----AAAGgCaUGUAAAAUGUUGCAA----------------------------------------------------------------------------A---A---GUAAcGUUuUACGcGCCUUAAC-A-------
```

6) Model for development of multicellular organisms

7) Powerful molecular genetic techniques

Genetic Markers



heterozygous ♀ hemizygous ♂

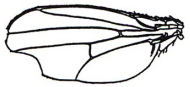
Bar (*B*) *X*
eye narrower than usual, oval shape

forked (*f*) *X*
bristles short with split or bent ends

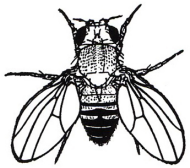
singed (*sn*) *X*
bristles short, gnarled, and wavy

Curly (*Cy*) *2nd*
wings curled upward instead of flat

Scutoid (*Sco*) *2nd*
missing bristles, especially from posterior thorax

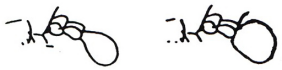


Serrate (*Ser*) *3rd*
(also called *Beaded-Serrate*, Bd^S)
wings notched



Dichaete (*D*) *3rd*
wings extended like jet plane instead of straight back

Stubble (*Sb*) *3rd*
bristles short and stubby

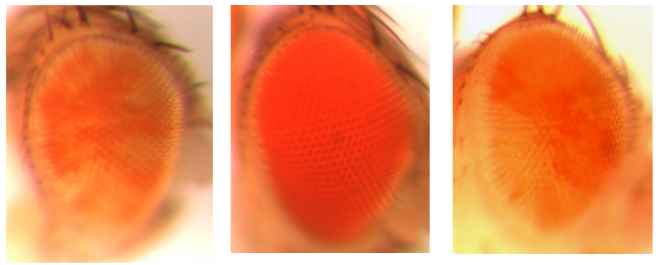


+/+ *Ubx*/+

Ultrabithorax (*Ubx*) *3rd*
haltere larger and rounder than normal

eyeless-Dominant (ey^D) *4th*
very small eyes

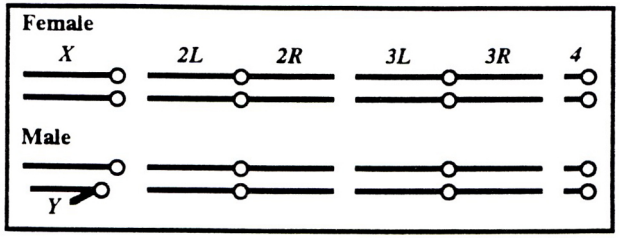
Eye Color

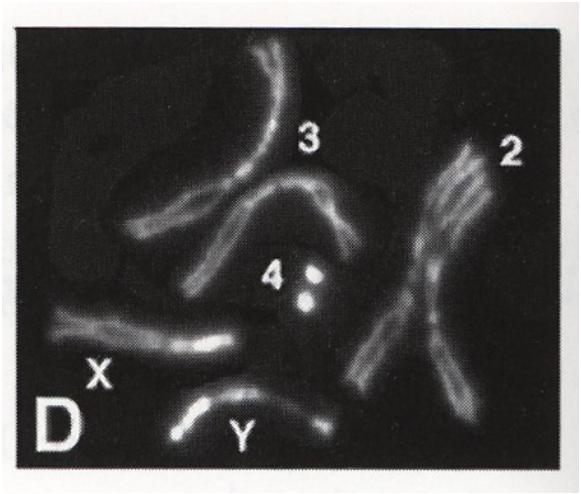


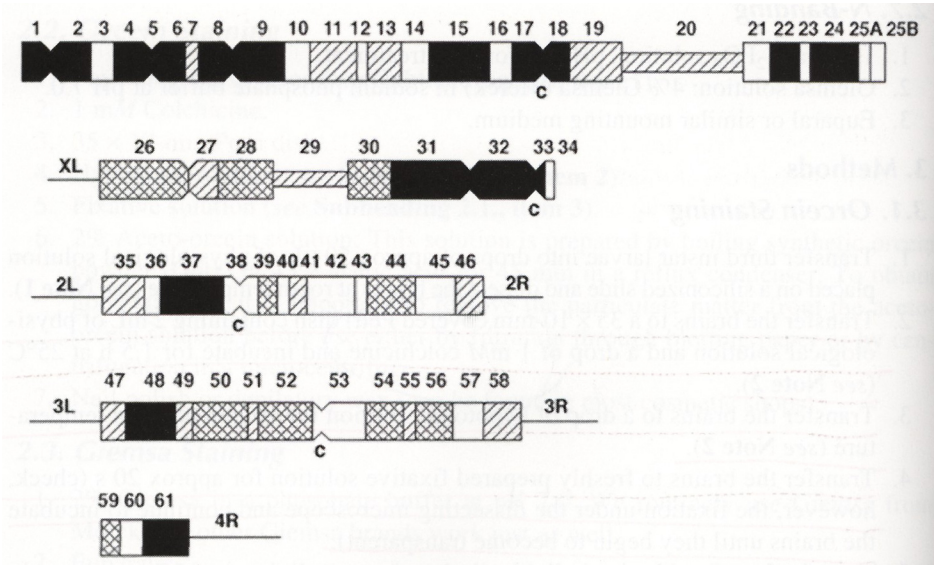
Bristle Length

8) Small genome (4 chromosome pairs)

Diploid Chromosome





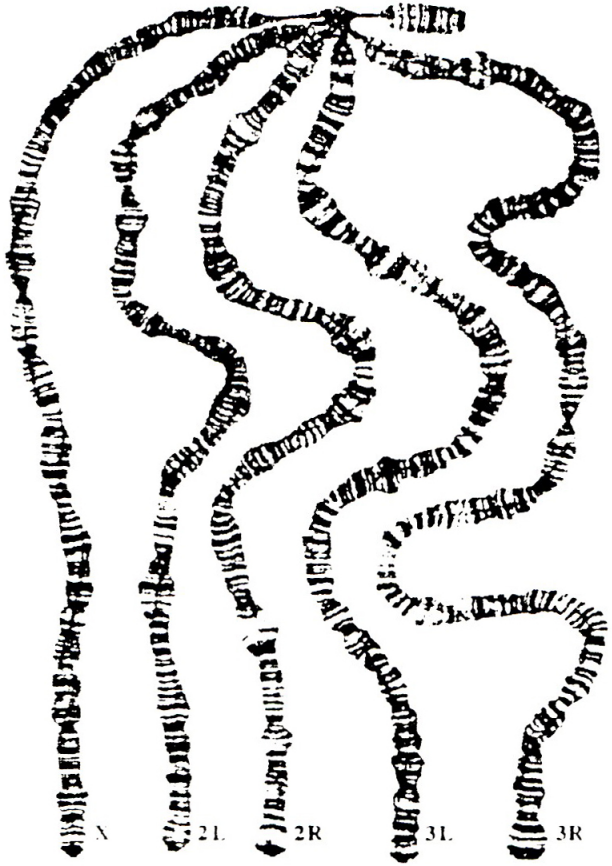


Heterochromatin

8) Small genome (4 chromosome pairs)

Polytene Chromosome

Salivary Gland Polytene Chromosomes

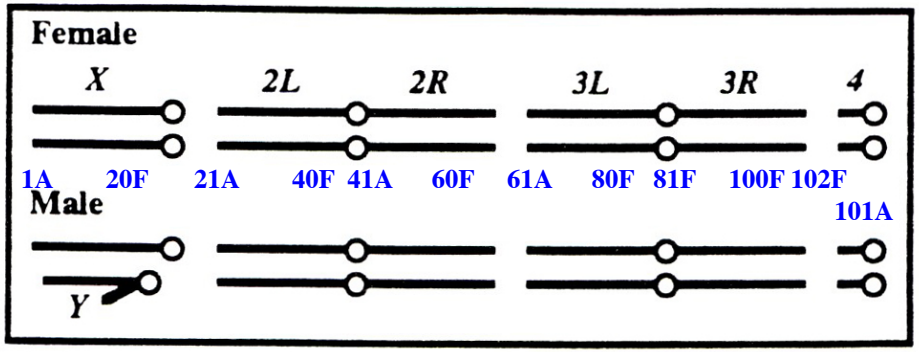


Each major chromosome arm is divided into 20 numbered segments:

X = segments 1-20
2L = segments 21-40
2R = segments 41-60
3L = segments 61-80
3R = segments 81-100
4th = segments 101-102

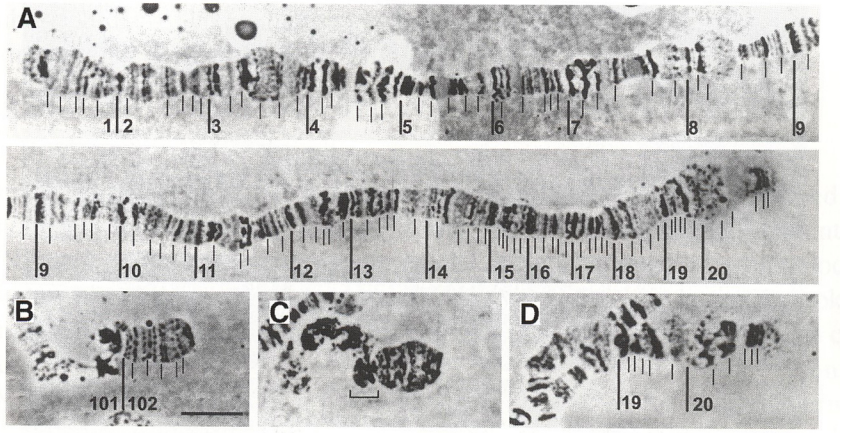
Segments are subdivided into lettered regions (A,B,C....)

Lettered regions contain bands (1,2,3,4,5,....)

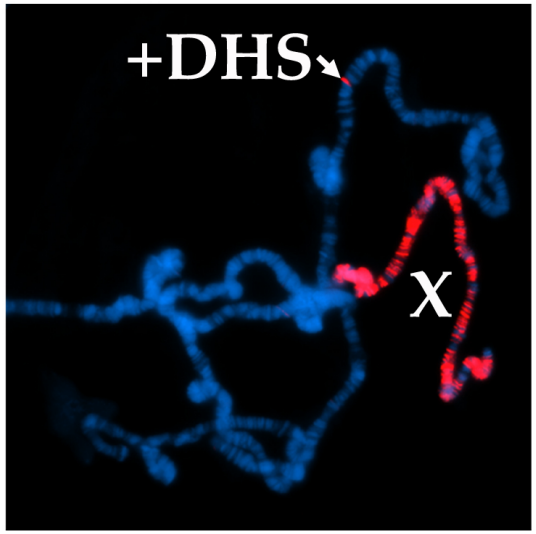


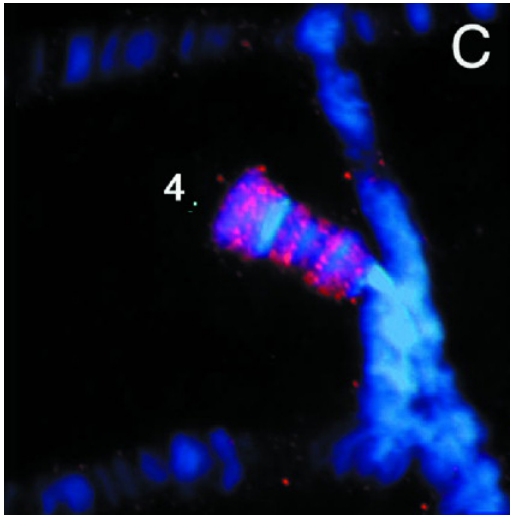
8) Small genome (4 chromosome pairs)

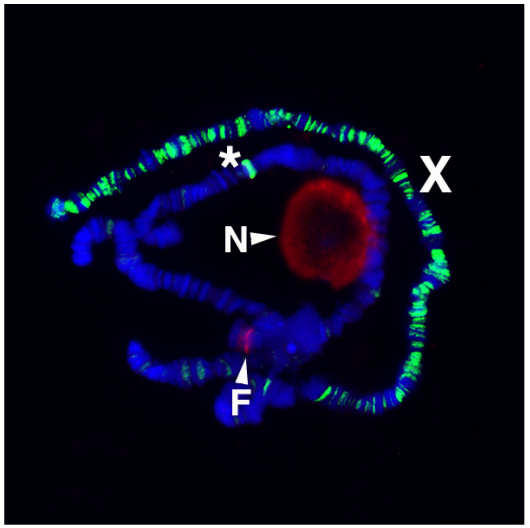
Cytological Map



Immunostaining







Nomenclature

1. Naming Genes

   - Gene names describe the loss-of-function phenotype.

     The *white* gene is required to make eyes red.

2. Recessive and Dominant Symbols

   - Genes named for recessive mutations are denoted with all lower case letters, such as the *white* gene or *w*.
   - Genes named for mutations that cause phenotypes in a dominant manner have the first letter capitalized and often do describe the phenotype.

     Ellipse (*Elp*) is a dominant allele of *torpedo*
   - Genes named after a protein product begin with an uppercase letter, such as the protein kinase C genes, *Pkc53E* and *Pkc98E*.

3. Allele Names

   - Allele names are indicated as superscripts to the gene name. For example, the first mutant allele of a gene is usually given allele name "1", such as w^1.
   - A wild-type allele is designated as "+", such as w^+
   - A generic mutant allele name is "-", such as w^-

4. Genotypes

   - The genotype of a chromosome is shown only if there is a mutation on it. They are listed in order:

     X/X; 2/2; 3/3; 4/4 for females

     X/Y; 2/2; 3/3; 4/4 for males
   - If only one genotype is shown for a chromosome then the fly is homozygous for this mutation.
   - Semicolons separate genotypes for each chromosome.

For example, the genotype:

$y\ w;\ cn\ bw\ ;\ hh^{n1}\ /\ TM3, Sb\ e$

indicates that the fly is :

- female
- homozygous for the X and 2nd chromosomes which carry two mutations each
- the third chromosome is heterozygous for a ts allele of *hedgehog* and the balancer *TM3*, which is marked by the dominant mutation Sb
- the fourth chromosome is wild-type (thus, the full genotype is not always shown)

Nomenclature

5. Other Notes

- Gene names are written in italic.
- Proteins are not in italics and are capitalized
- Fusion genes have a double colon, e.g., *Antp::ftz*.
- The genotype of transposons is indicated within braces, such as *P{w+, lacZ}*.

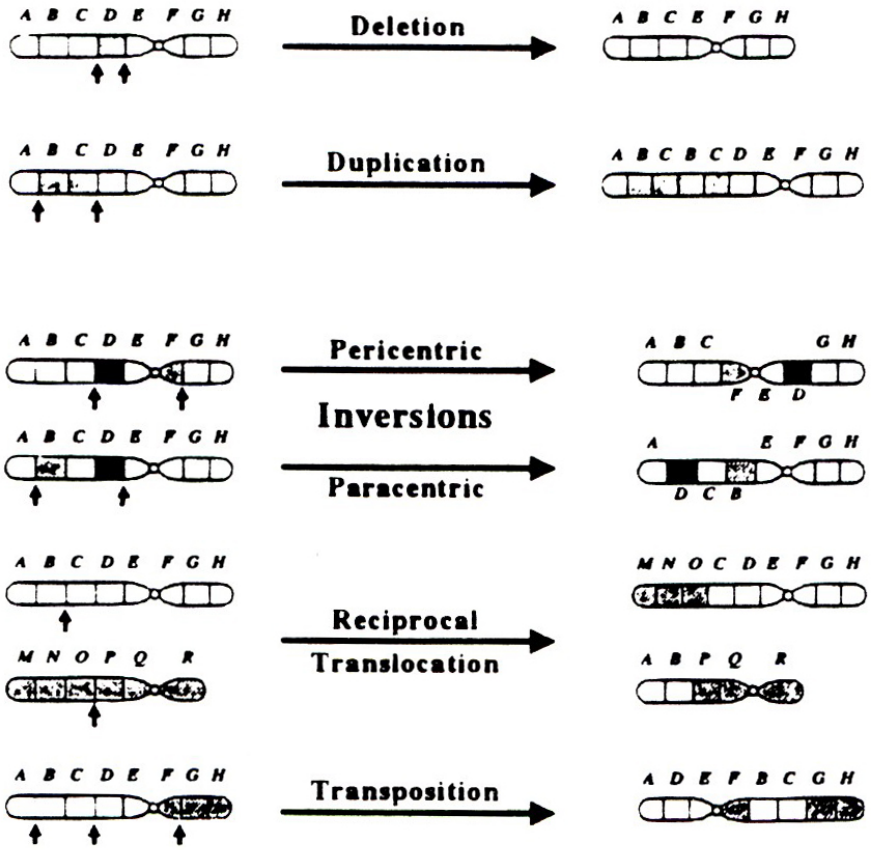
Sex Determination by X:A ratio

1:1 is female (2X:2A) and 0.5:1 is male (X:2A)

XX/Y:2A ?, X/O:2A ?

Nomenclature

Common Chromosome Rearrangements



Chromosomal rearrangement designations:

Df(3R) = deletion within the right arm of the 3rd chrom.

Dp(1;3) = duplication of a part of the X onto the 3rd

In(2L) = inversion in left arm of the 2nd chromosome. Does not contain the centromere (paracentric)

In(2LR) = inversion contains the centromere (pericentric)

T(2;3) = reciprocal translocation between the 2nd and 3rd

Tp(3) = transposition in 3rd

Balancer Chromosome

- Unlike yeast, Drosophila is an obligate diploid: meiosis occurs every generation.

  How are lethal mutations maintained?

- 1) Balancers were identified as "crossover suppressors." **(No recombination)**

- Balancers contain multiple inversions.

- Crossovers between normal and inverted chromosomes produce lethal gametes and are not recovered.

- 2) Balancers contain recessive lethal mutations.

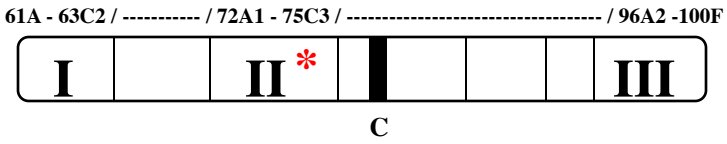
- 3) Balancers have dominant, visible mutations.

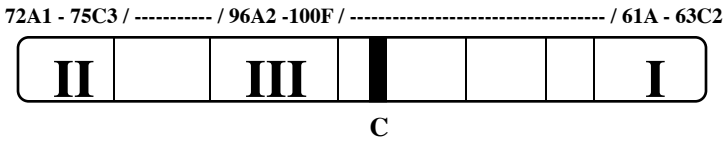
- X chromosome balancers do not have recessive lethals. Why?

  How does one balance a recessive lethal on the X?

- No recombination in mates or chromosome 4

WT-3rd





Balancer-3rd

2nd chr Balancer (*CyO*)

WT: black body, red eye, straight wing

***y*: yellow body, *pr*: purple eye, *CyO*: curled wing**

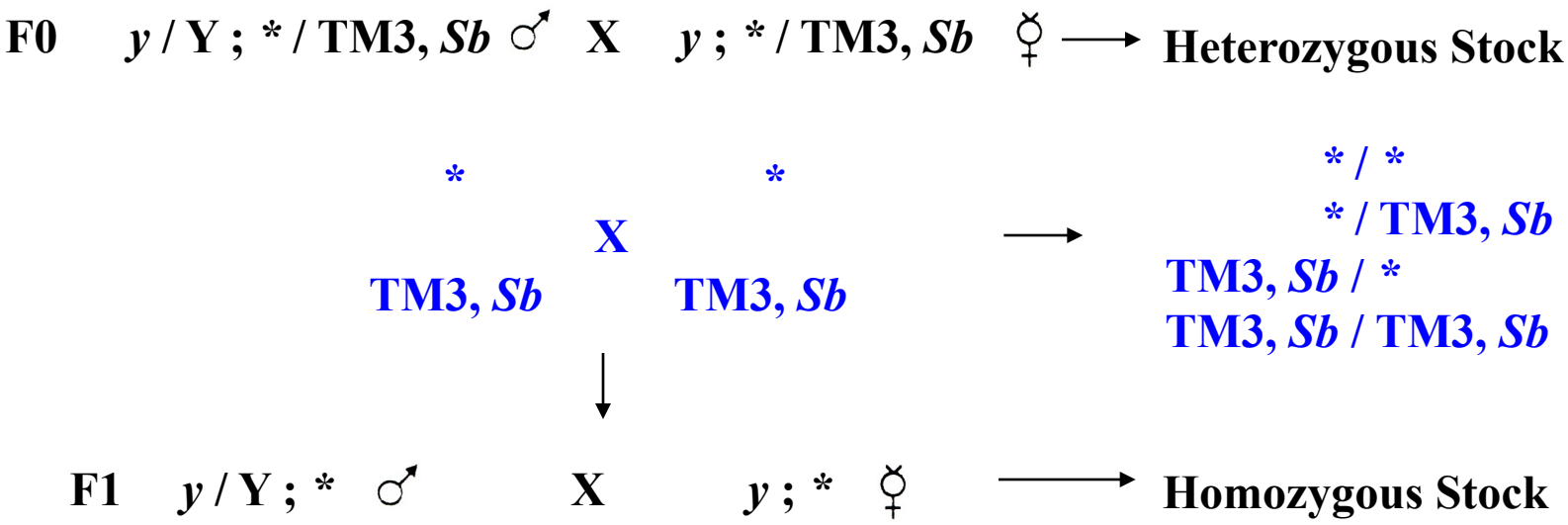
F0 y / Y ; pr / CyO ♂ **X** y ; pr / CyO ♀ ⟶ **Heterozygous Stock**

	pr		*pr*	*pr / pr* *pr / CyO* *CyO / pr* *CyO / CyO*
	CyO	X	*CyO*	⟶
	↓			
F1	y / Y ; pr ♂	**X**	y ; pr ♀	⟶ **Homozygous Stock**

3rd chr Balancer (TM3, *Sb*)

WT: black body, long bristle

***y*: yellow body, *: unknown mutant, *Sb*: short bristle**



Cancer Study in the Fruitfly

1. Why is fly used for study of human cancer?
2. How is cancer studied in fly?
3. How are cancer genes found in fly?
4. What kind of cancer genes were discovered in fly?

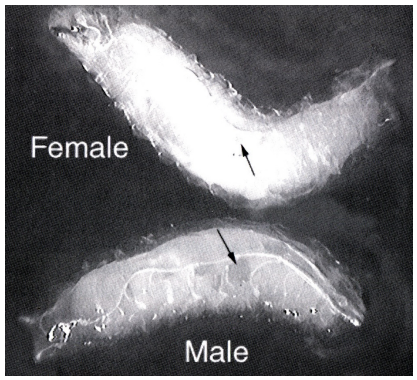
Advantage of fly to study cancer

1) Evolutionarily conserved simpler pathway of cancer
- less redundant cancer genes

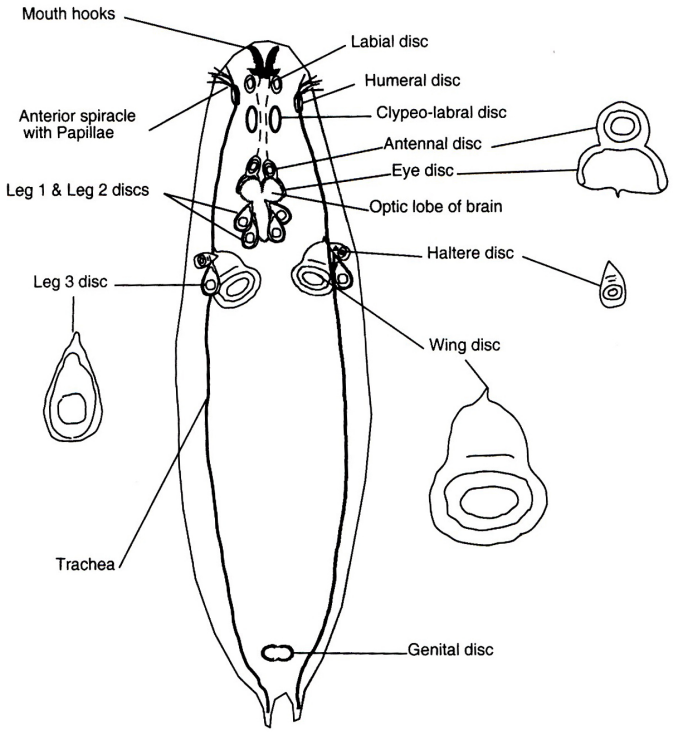
2) Powerful genetic screening of cancer genes in animal model
- loss-of-function or gain-of-function mutants
- forward genetics or reverse genetics

Fly tissues for tumor analysis

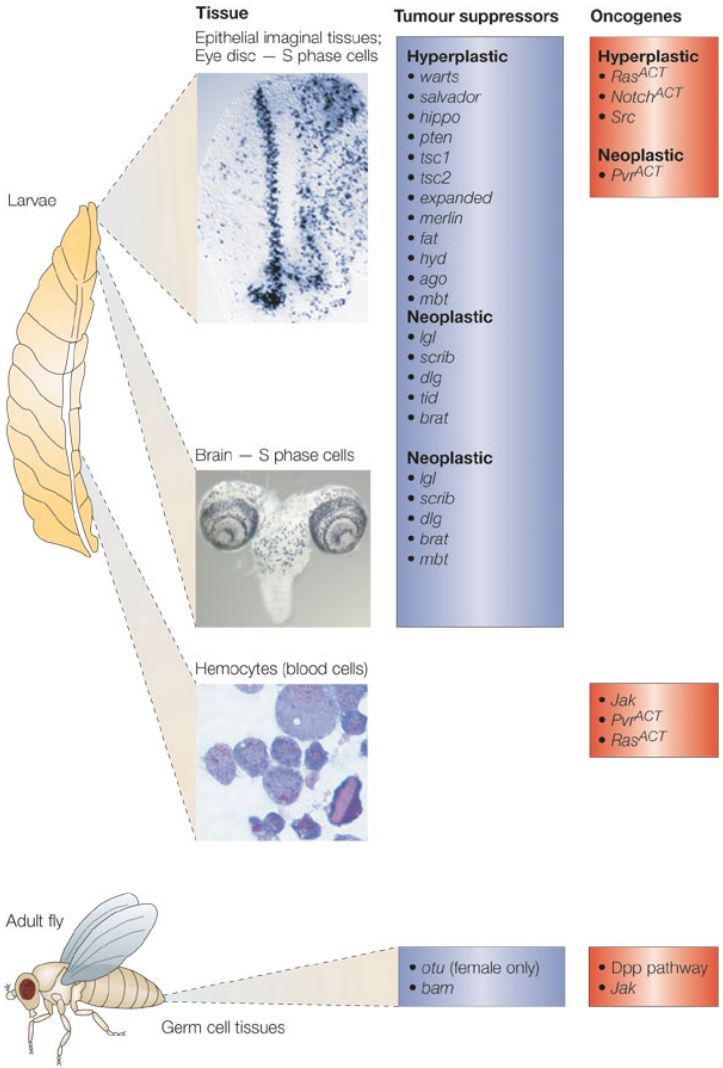
Larva



Imaginal disc

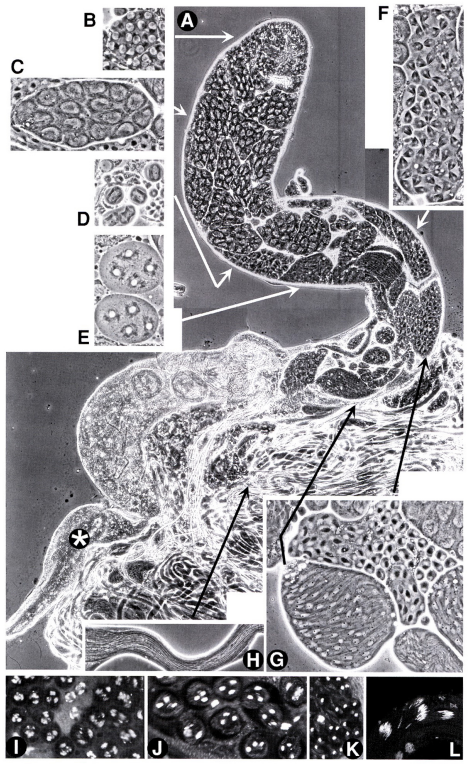


Cancer genes

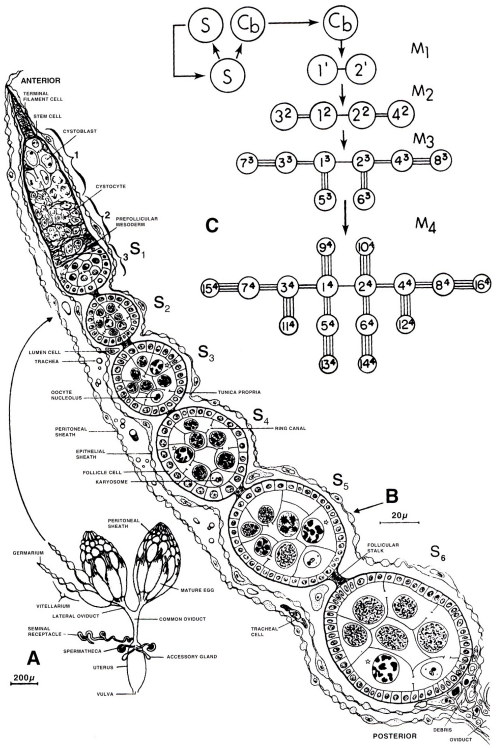


Fly tissues for tumor analysis

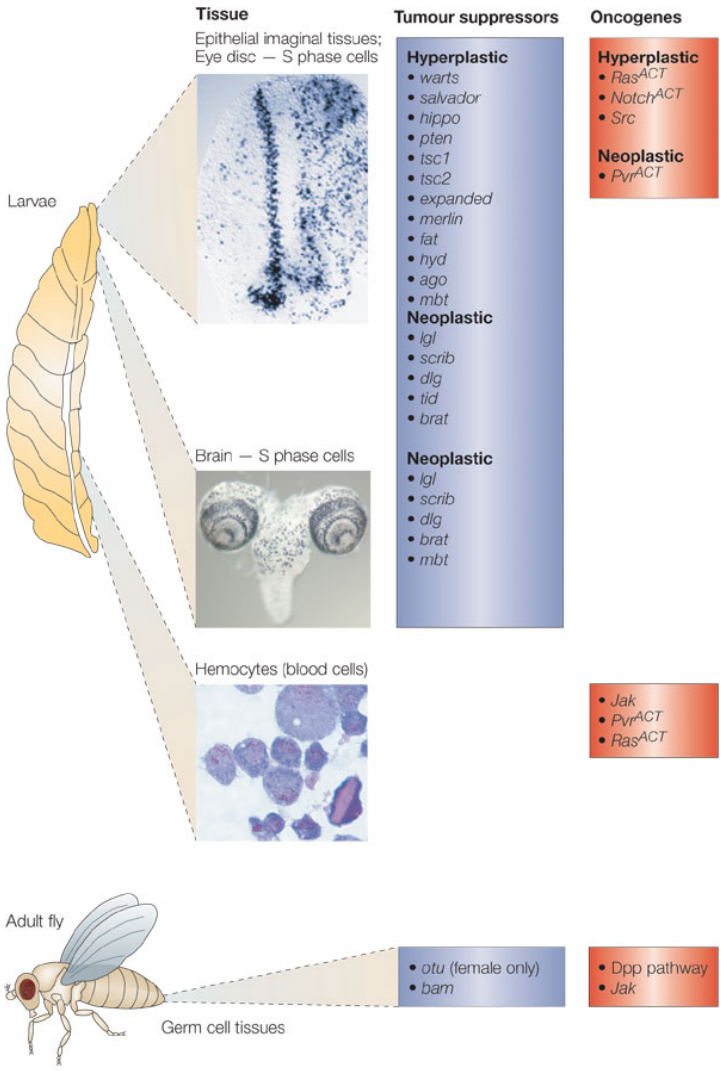
Testes



Ovary



Cancer genes



Mutagenesis for Cancer study

1) EMS

- single (or several) bases substitution and deletion

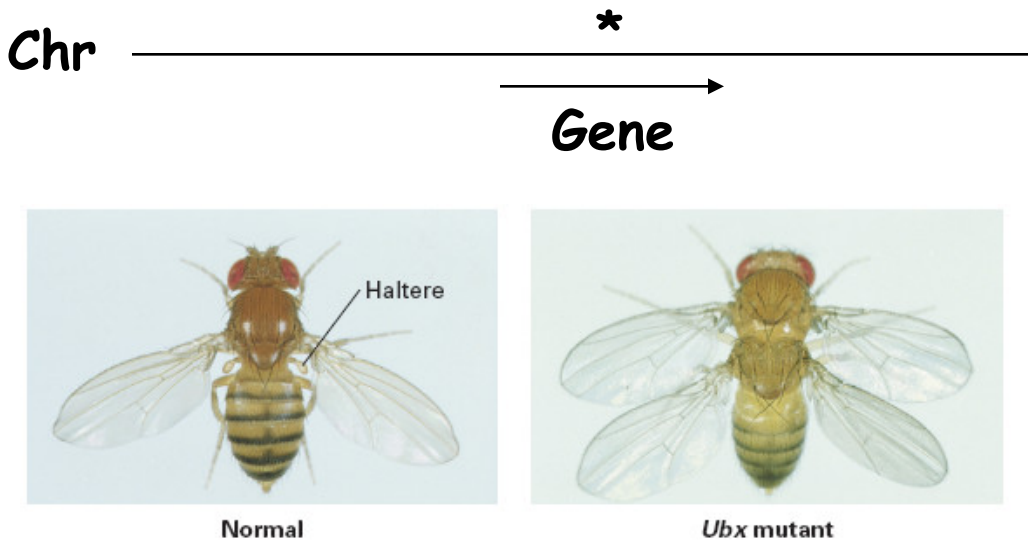
2) X-rays

- chromosome rearrangements

3) P-element

- insertion or deletion

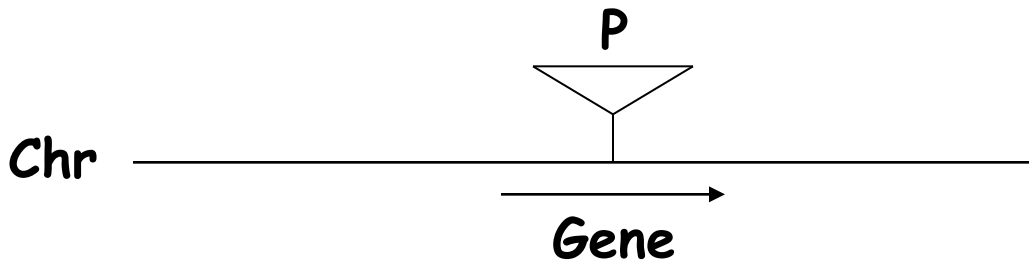
EMS Mutagenesis (Point mutation)



Mutation Mapping

1. Chromosome Mapping by Balancer
2. Linkage Mapping with known mutations
3. Deficiency Mapping

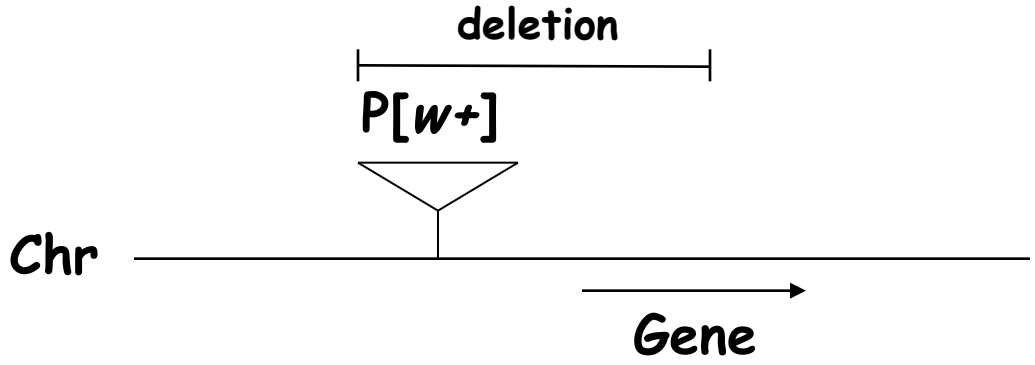
P-element Mutagenesis (Insertion)



Mutation Mapping

1. Chromosome Mapping by Balancer
2. Cytological Mapping in Polytene chromosome
3. Genomic Inverse PCR

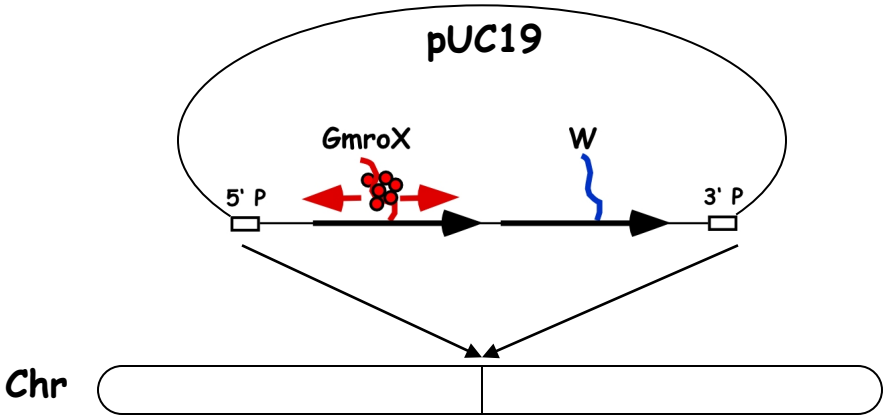
P-element Mutagenesis (Deletion)



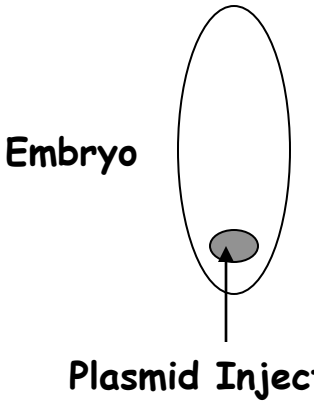
Mutation Mapping

1. Selection of deletion (w/o *w+*)
2. Genomic Southern
3. Genomic Inverse PCR

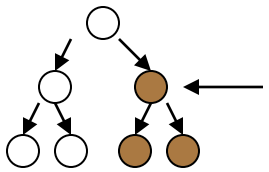
Transgene Construction



+ Transposase



Meiosis



Genetic manipulation of fly for the discovery of cancer genes

1) Genetic screening of loss-of-function
- EMS, P-element insertion

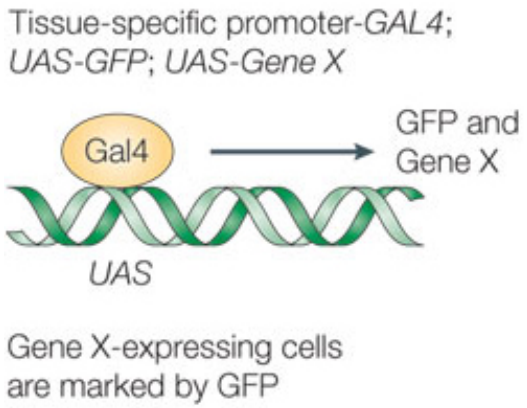
2) Genetic screening of modifier gene
- genetic pathways
- suppressor mutant: *su(m)*

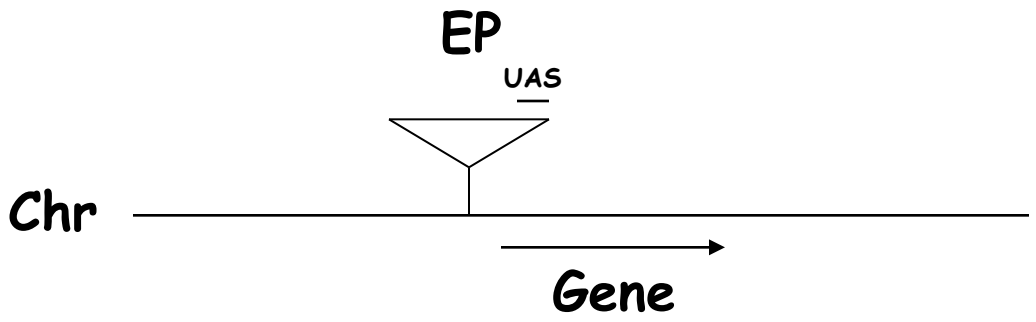
su(w^a) : recessive suppressor of *w^a* (apricot eye color),
su(w^a) w^a : brown eye

- enhancer mutant: *e(m)*

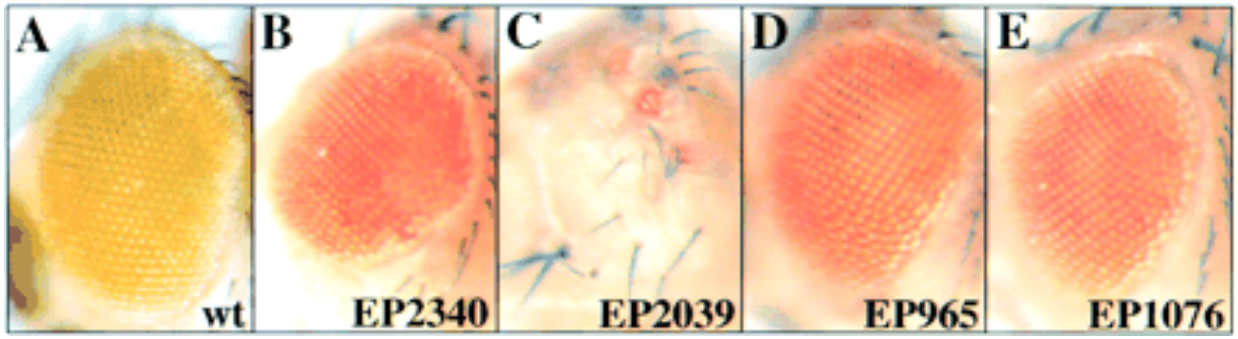
e(w^e) : recessive enhancer of *w^e* (eosin eye color),
e(w^e) w^e : white eye

3) gain-of-function
- GAL4-UAS system (overexpression of gene)



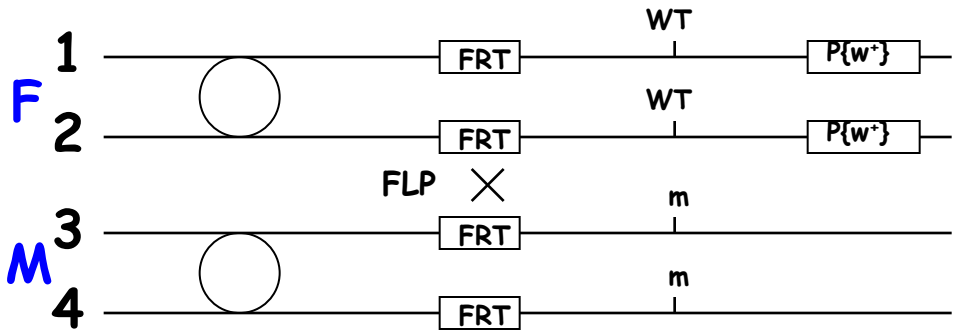


ey-GAL4; UAS-gene



"An overexpression screen in *Drosophila* for genes that restrict growth or cell-cycle progression in the developing eye" Tseng & Hariharan, Genetics 2002

4) Genetic screening using clonal (mosaic) analysis -FLP/FRT recombination

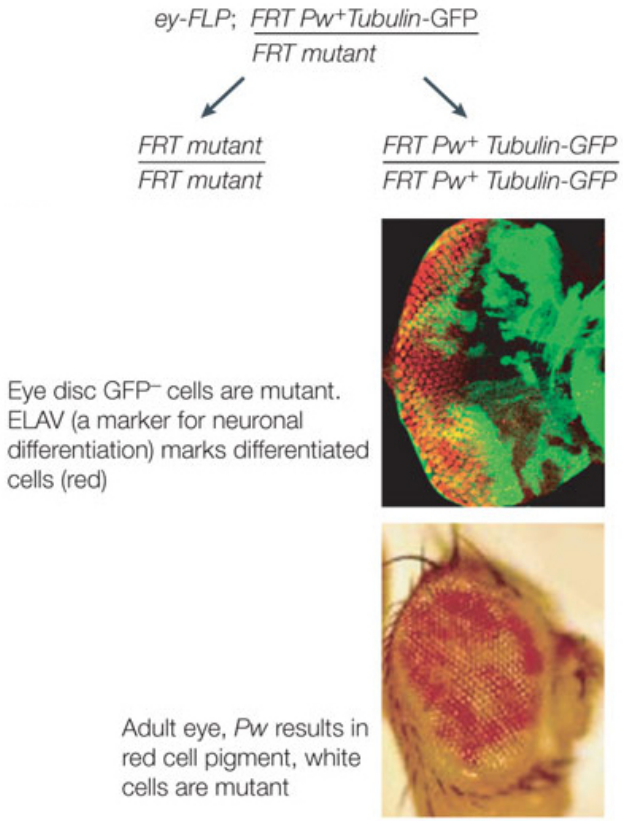


Re -

1+3 : WT *P{w+}* / m
1+4 : WT *P{w+}* / m
2+3 : WT *P{w+}* / m
2+4 : WT *P{w+}* / m

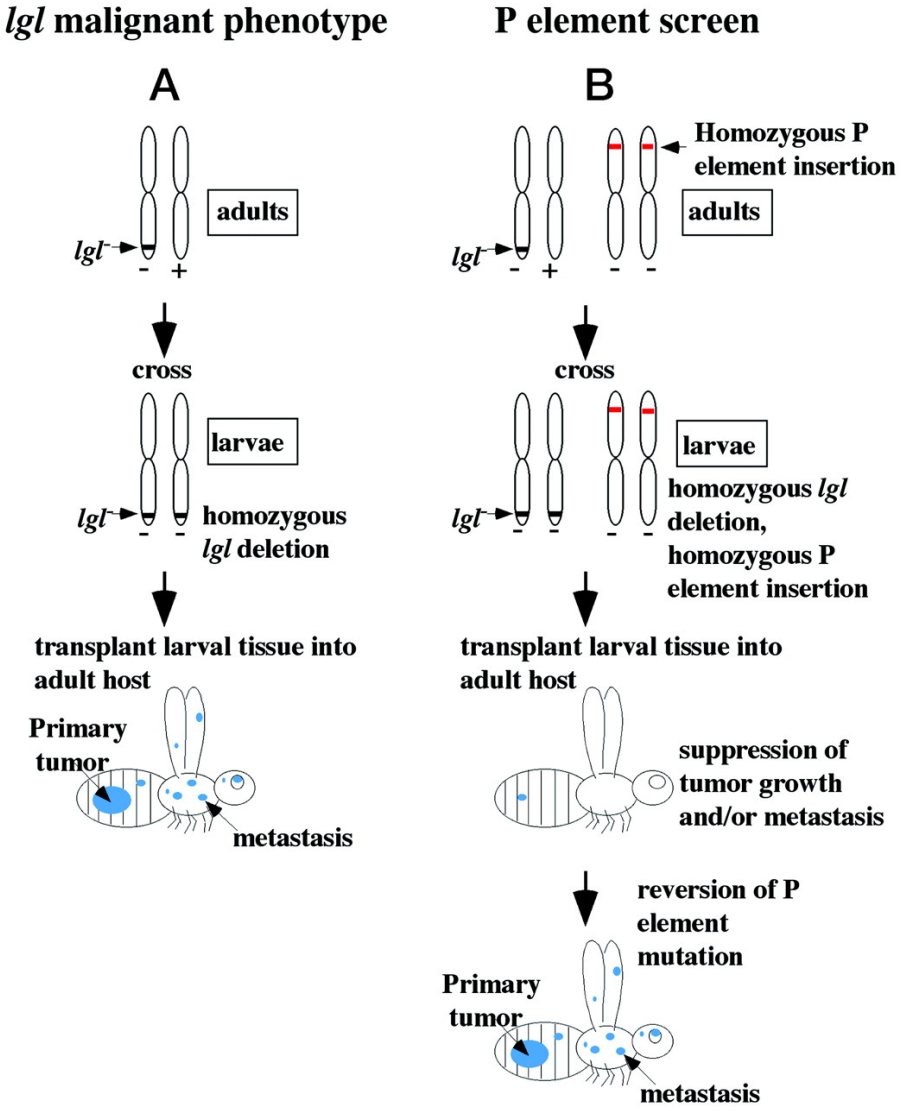
Re +

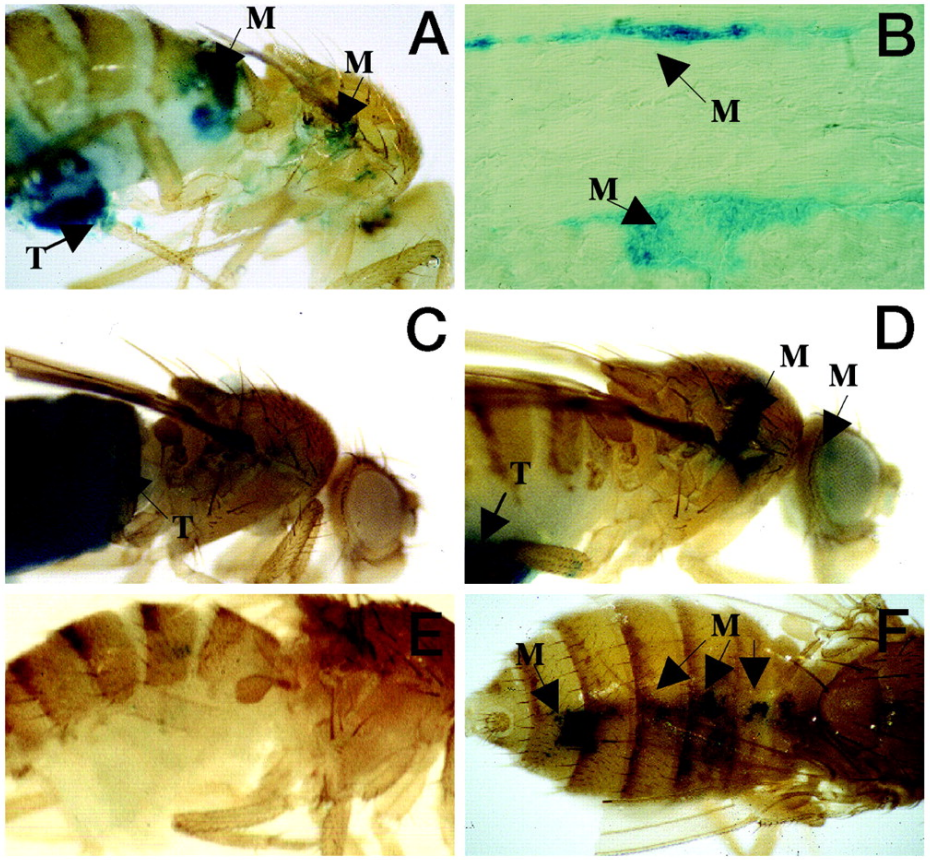
1+3 : WT *P{w+}* / WT *P{w+}*
1+4 : WT *P{w+}* / m
2+3 : m / WT *P{w+}*
2+4 : m / m



"Archipelago regulates Cyclin E levels in Drosophila and is mutated in human cancer cell lines" Moberg et al., Nature 2001

5) Genetic screening in invasion/metastasis -transplantation assays





"*Drosophila* screening model for metastasis: Semaphorin 5c is required for l(2)gl cancer phenotype." Woodhouse et al., PNAS 2003

Four hallmarks of cancer in fly

1) Self-sufficiency in growth/proliferation signals
   (Cell growth/proliferation)

2) Evading apoptosis
   (Survival)

3) Insensitivity to anti-proliferative signals
   (failure of differentiation)

4) Tissue invasion/metastasis
   (Transplantation)

Fly genes to show hallmarks of cancer

Oncogenes

Drosophila gene/pathway	Self sufficiency in growth/ proliferation signals	Evading apoptosis	Resistance to antiproliferative signal (differentiation)	Tissue invasion and metastasis
Oncogenes *	Effect of upregulation			
Cyclin E (activator of the G1-S phase Cdk2 protein kinase)[2,3]	Yes, drives entry into the cell cycle	No, increased cell death	No	NE
E2f1 (transcription factor of S phase genes)[4-6]	Yes, drives entry into the cell cycle	No, increased cell death	No	NE
Cyclin D (activator of the G1-S phase Cdk4 protein kinase)[7] with Cdk4	Yes, increases cell growth and cell proliferation	No, but proliferation can ovrride cell death in the eye	No	NE
Myc (HLH transcription factor) with *Max* (Myc binding HLH protein)[8,9]	Yes, increases cell growth and increases Cyclin E protein and S phase entry	No, but proliferation can override cell death in the eye	No	NE
bantam (microRNA)[10,11] - human homolog unknown	Yes, increased cell growth and proliferation	Yes, inhibits apoptosis	No	NE
diap1 (apoptosis inhibitor)[12,13]	No	Yes, inhibits apoptosis	No	NE, but promotes ovarian border cell migration
buffy (BCL2 anti-apoptotic)[14]	No, cell proliferation is inhibited	Yes, inhibits apoptosis	No	NE
Receptor Tyrosine Kinase-Ras-MAPK pathway[9,15-21]	Yes, increases Cyclin E protein levels.	Yes, inhibits apoptosis by inhibiting the expression and activity of the cell death inducer, Hid	No, promotes differentiation.	NE, but required for germ cell migration. Does not cause invasion of eye disc cells
InR-PI3K pathway[22-28]	Yes, hyperplastic overgrowth	No, cell death still occurs when AKT is expressed in the eye disc, but proliferation can override cell death	No, promotes differentiation.	NE
JAK-STAT pathway[29-33]	Yes, hyperplastic overgrowth of imaginal disc epithelium	?	Yes, prevents differentiation of germ cells, but promotes differentiation of hemocytes and differentiation occurs in the eye disc	NE, but important for specifying ovarian border cell and tracheal cell fate, and required for germ cell migration.
DPP (TGFβ) pathway[34-38]	Yes, hyperplastic overgrowth.	Yes, decreased cell death in	Yes, prevents	NE, but loss-of-function

Fly genes to show hallmarks of cancer

Oncogenes

DPP (TGFβ) pathway[34-38]	Yes, hyperplastic overgrowth. But can also inhibit cell proliferation in some contexts[39].	Yes, decreased cell death in the wing disc	Yes, prevents differentiation of germ cells, but promotes differentiation in the eye disc. Blocked by Rb in the wing disc.	NE, but loss-of-function can result in changes in cell shape and cytoskeleton[40,41].
Hedgehog (HH) pathway[42,43]	Yes, hyperplastic overgrowth. Increases Cyclin E and Cyclin D levels	?	No, promotes differentiation	NE
Wingless (WG) pathway[44-46]	Yes, hyperplastic overgrowth. But can also inhibit cell proliferation in some contexts[47].	No, cell death occurs later in the eye disc	Yes, prevent differentiation in the eye disc	NE
Notch pathway[19,30,44,45,48-50]	Yes, hyperplastic overgrowth. But can also inhibit cell proliferation in some contexts[47].	No, cell death occurs later in the eye disc	Yes, prevents differentiation in the eye disc	NE
Pvr (PDGF/VEGF) pathway[32,51-53]	Yes, neoplastic overgrowth of wing imaginal tissue and hemocytes	?, in wing discs Yes, promotes hemocyte cell survival	Yes, prevents differentiation of wing discs, but not of hemocytes	NE, but probably yes? Wing imaginal disc cells lose polarity. Required for guidance in border cell and hemocyte migration.
FGFR (Heartless/ Breathless) pathway[54,55]	?	?	?	NE, but promotes migration of embryonic mesoderm, border cells and tracheal cells
JNK pathway[56]	?	No, increased cell death	No, promotes hemocyte differentiation	NE, promotes cell shape changes in many tissues
Src (tyrosine kinase)[57,58]	Yes, hyperplastic overgrowth	No, a dose-dependent increased cell death in the eye disc	No, differentiation eventually occurs	NE
slik (Sterile-20 kinase), MAPKKK cascase[59,60]. Human homologues, LOK and SLK[61]	Yes, hyperplastic overgrowth	No, increased cell death	No	NE

Fly genes to show hallmarks of cancer

Tumor suppressors

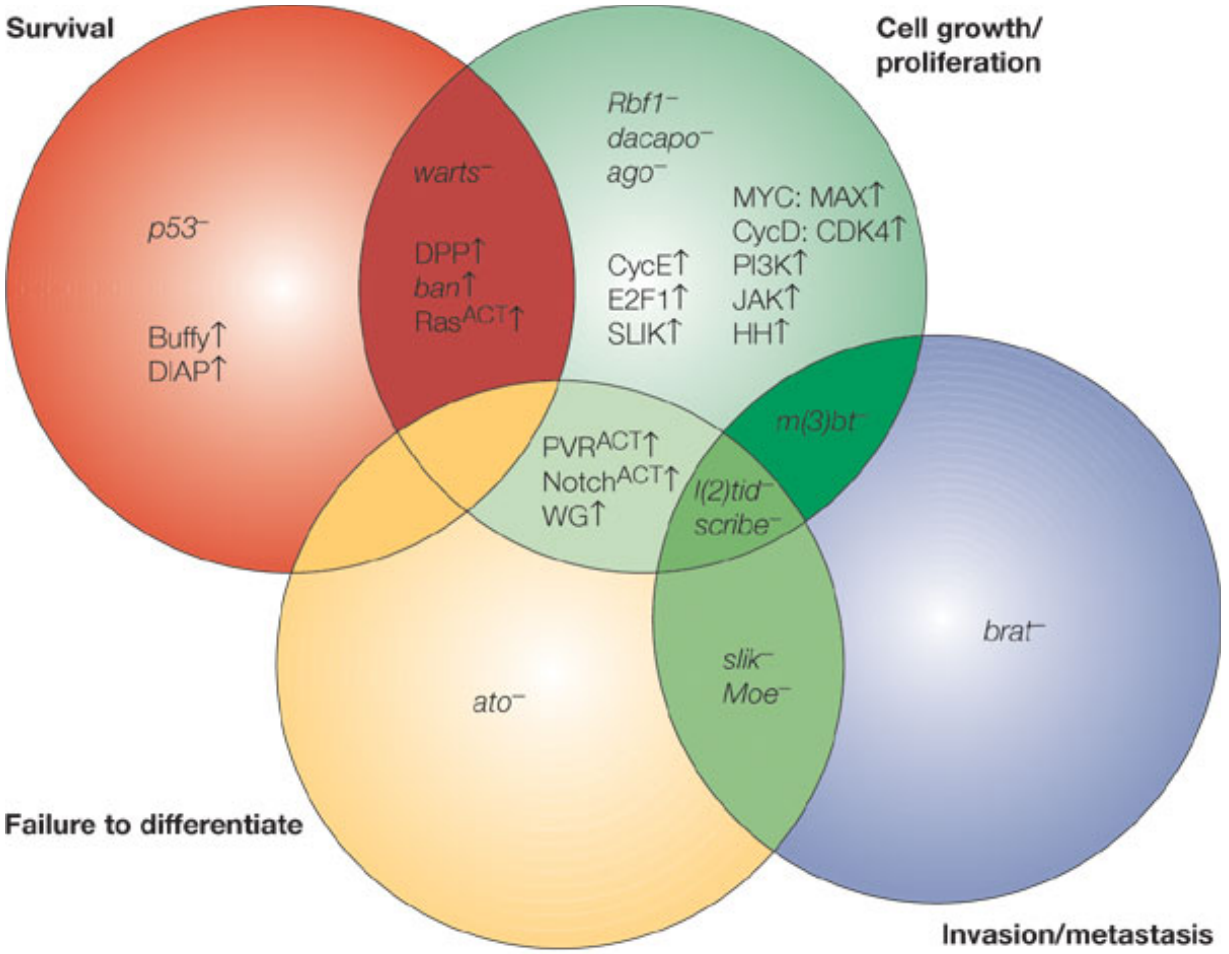
Drosophila gene/pathway	Self sufficiency in growth/ proliferation signals	Evading apoptosis	Resistance to antiproliferative signal	Tissue invasion and metastasis
Tumour suppressors **	**Effect of mutation**			
dacapo (p21, p27 homologue) – inhibitor of Cyclin E/CDK2[62,63]	Yes, increased proliferation	Probably No?	No	NE
Rbf1 (Retinoblastoma homologue) - repressor of E2F1[64,65]	Yes, increased cell growth and proliferation	Probably No?	No	NE
p53 - transcription factor[66]	No	Yes, decreased cell death	No	NE
ago (cdc4, Fwb7 homologue) - F-box ubiquitin ligase subunit, mutated in human cancers[67-69]	Yes, increased stability of Cyclin E and Myc. Upregulation of the Notch pathway	?	No	NE
warts (protein kinase)/*sav* (WW domain)/*hippo* (Mst family protein kinase) pathway[70-79]. *warts* and *sav* homologues are mutated in human cancers[76,80]	Yes, increased levels of Cyclin E	Yes, increased levels of Diap1 (apoptosis inhibitor)	No	NE
hyd (hyperplastic discs) - HECT domain ubiquitin ligase. Human homologue, *EDD*, may be involved in cancer[81,82]	Yes, hyperplastic overgrowth	?	No	NE
Pten - lipid phosphatase (see PI3K pathway above)	Yes, hyperplastic overgrowth by activation of the PI3K signalling pathway	(see PI3K pathway above)	(see PI3K pathway above)	(see PI3K pathway above)
Tsc1/Tsc2 (*gigas*) - Hamartin/Tuburin complex (see PI3K pathway above)	Yes, hyperplastic overgrowth by activation of the PI3K signalling pathway	(see PI3K pathway above)	(see PI3K pathway above)	(see PI3K pathway above)
fat - atypical cadherin[81]	Yes, hyperplastic overgrowth	?	No	NE
expanded/Merlin (NF2) - FERM domain proteins[81,83]	Yes, hyperplastic overgrowth	?	No	NE
Moesin (*Moe*)-ERM	?	?	?	NE, but wing disc cells

Fly genes to show hallmarks of cancer

Tumor suppressors

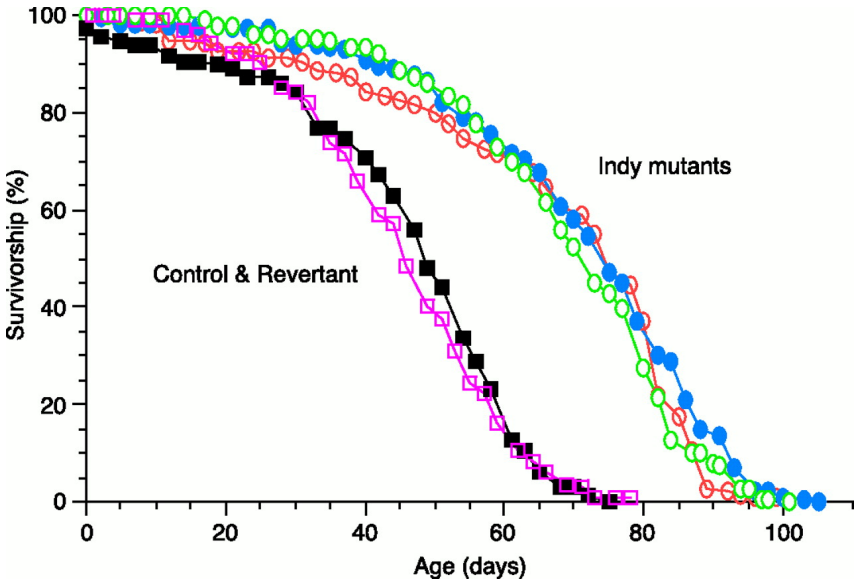
Moesin (*Moe*)-ERM protein[84]. Human homologues, Ezrin, Radixin and Moesin.	?	?	?	NE, but wing disc cells lose polarity and become migratory
slik (Sterile-20 kinase), MAPKKK cascase[59,60] Human homologues, LOK and SLK[61]	?	No, increased cell death	Yes, shows a partial differentiation defect	NE, but shows loss of epithelial polarity and increased migration
otu (*ovarian tumour*) - tudor domain DNA binding protein[81] – novel protein.	Yes, neoplasia of germ cells	?	Yes, remain as stem cells	?
bam (*bag-of-marbles*) - novel protein[81]	Yes, neoplasia of germ cells	?	Yes, remain as stem cells	?
scrib (LAP4)/*dlg1* (MAGUK) /*l(2)gl* (WD40 domain). Human homologues, *hSCRIB*, *DLG1-4*, *LGL1-2* may have roles in cancer[19,85-87]	Yes, neoplastic overgrowth of brain and imaginal tissue from homozygous mutant larvae, upregulation of Cyclin E in eye imaginal disc mutant clones	No, increased cell death in clones	Yes, when homozygous and in clones in the eye imaginal disc	Yes, brain tissue from homozygous mutant larvae invades adjacent tissue and to distant sites
l(2)tid (*tumourous imaginal disc*) - DnaJ family chaperone. Human homologue, *hTID1* implicated in cancer[81,88,89]	Yes, neoplastic overgrowth of imaginal discs	?	Yes, differentiation defects in imaginal discs	Yes, imaginal discs from homozygous mutant larvae invades in transplantation assays
brat (*brain tumour*) - translational repressor and inhibitor of cell growth. Putative human homologue, *Trim3* (*BERP*), not yet implicated in cancer[81,87,90].	Yes, neoplastic overgrowth of the larval brain, but imaginal discs are not overgrown in homozygous mutants.	?	Yes, ganglion mother cells cannot form neurons	Yes, brain and imaginal tissues from homozygous mutant larvae invades in transplantation assays
l(3)mbt (*malignant brain tumour*) – Polycomb group chromatin factor. Human homologue, *L3MBTL* implicated in cancer[81,91]	Yes, neoplastic overgrowth of the larval brain, and hyperplastic overgrowth of imaginal discs	?	Yes, ganglion mother cells cannot form neurons	Yes, brain tissue from homozygous mutant larvae invades in transplantation assays

Hallmarks of cancer in fly imaginal discs



"USING *DROSOPHILA MELANOGASTER* TO MAP HUMAN CANCER PATHWAYS"
Brumby AM and Richardson HE, Nat. Rev. Cancer. 2005. 5:626-39.

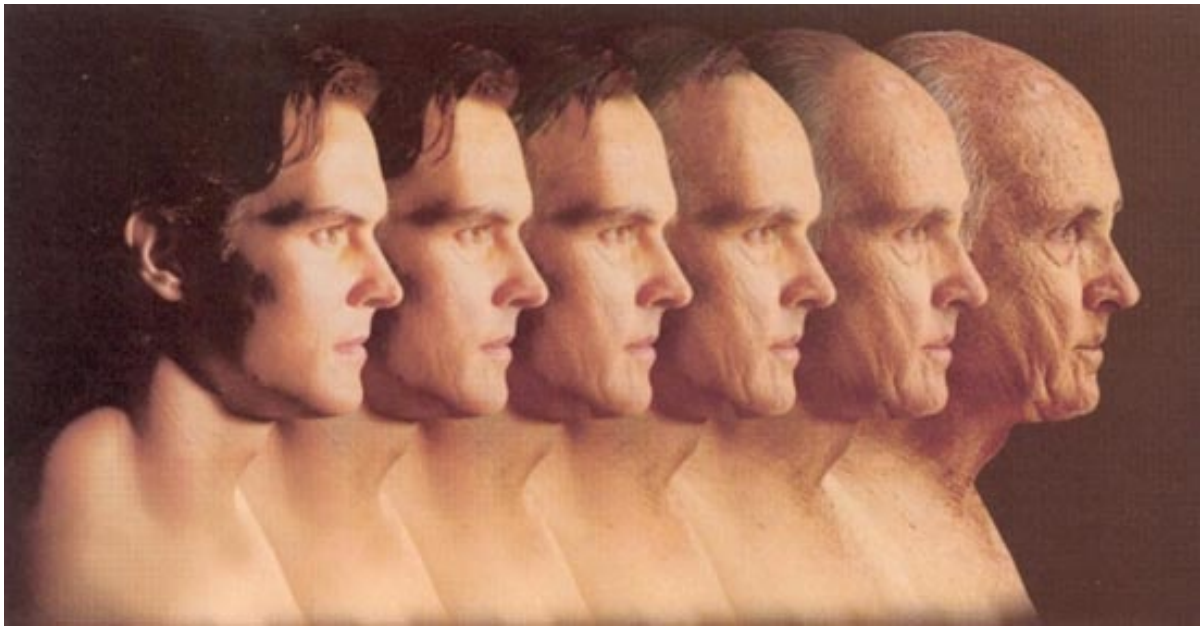
Studies of aging disease in fly



How is aging processed?

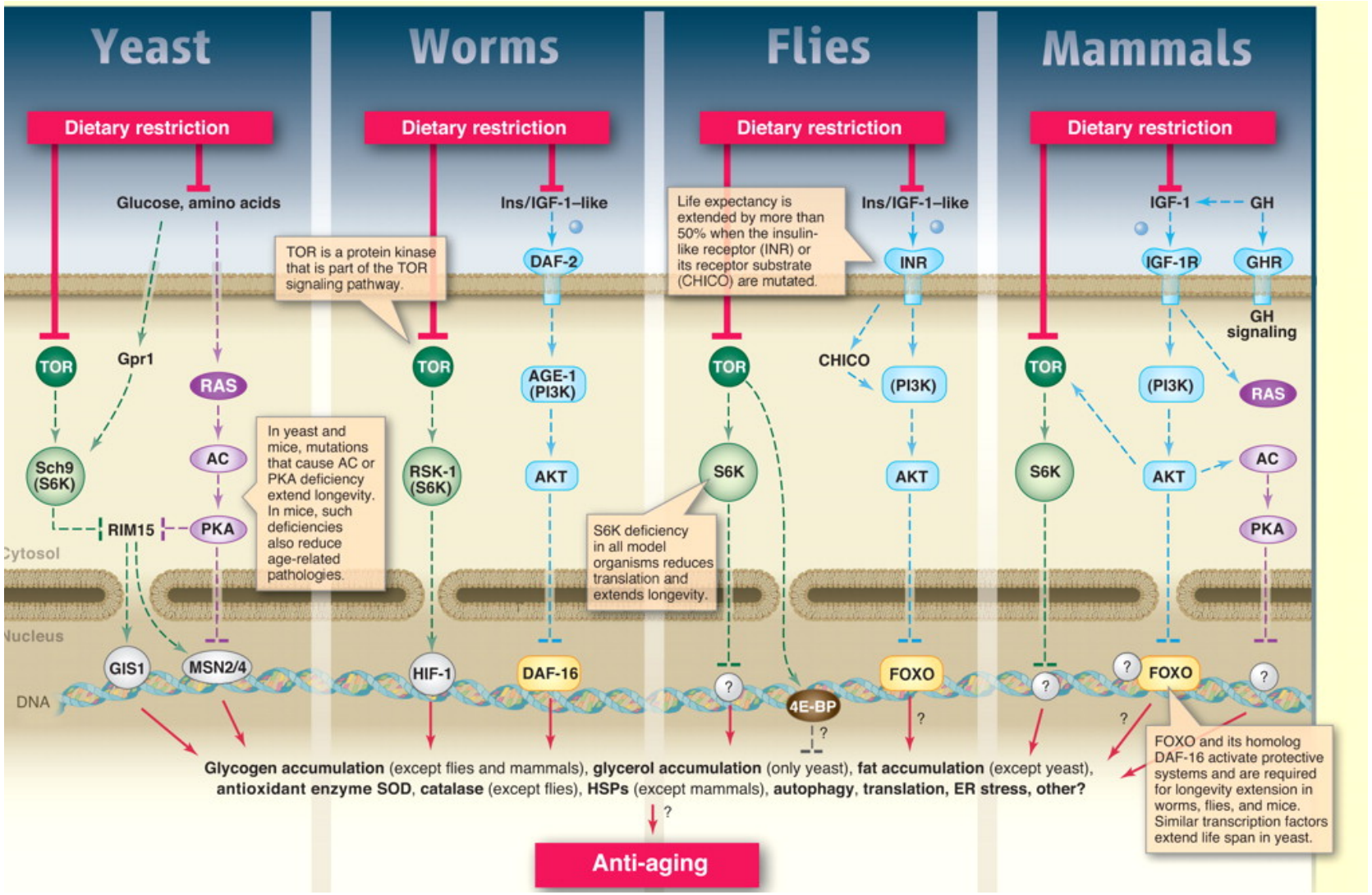
???

Aging



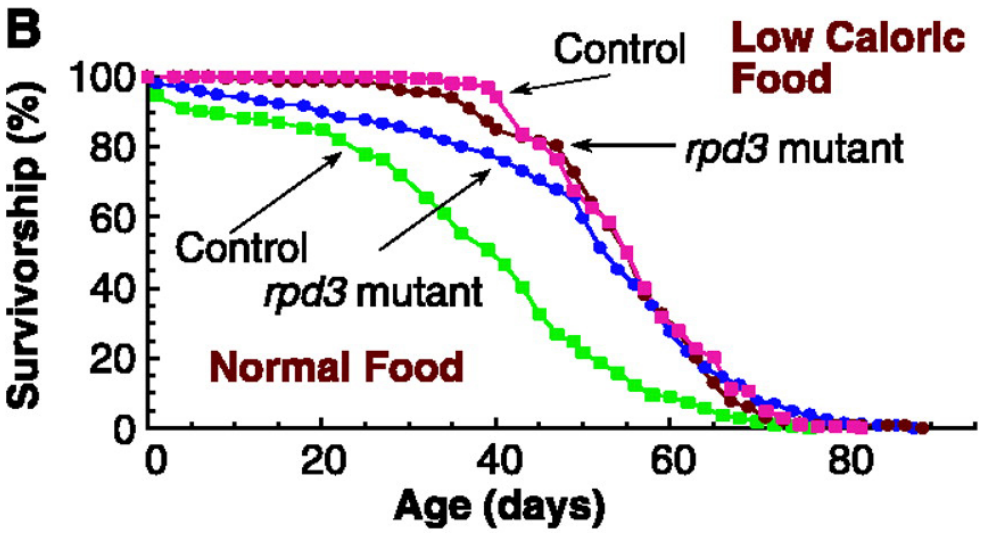
Longevity mechanisms are evolutionarily conserved in several species.

Insulin/IGF-1, TOR-S6K, Sir2 signaling pathways



Fontana et al., Science 2010

Dietary restriction extends life-span in several species.



Rogina et al., Science 2002

Before starting dietary restriction (81.6 kg)

After 7 years of dietary restriction (60.8 kg)

Fontana et al., Science 2010

Aging Study in the Fruitfly

The benefits of using *Drosophila* for studying aging

1) Its relatively short life span (3 months) compared to mammalian

2) Large number of progeny (200 progenies per female)

3) Diverse developmental stages compared to C.elegans and yeast

4) Ease of manipulating genes

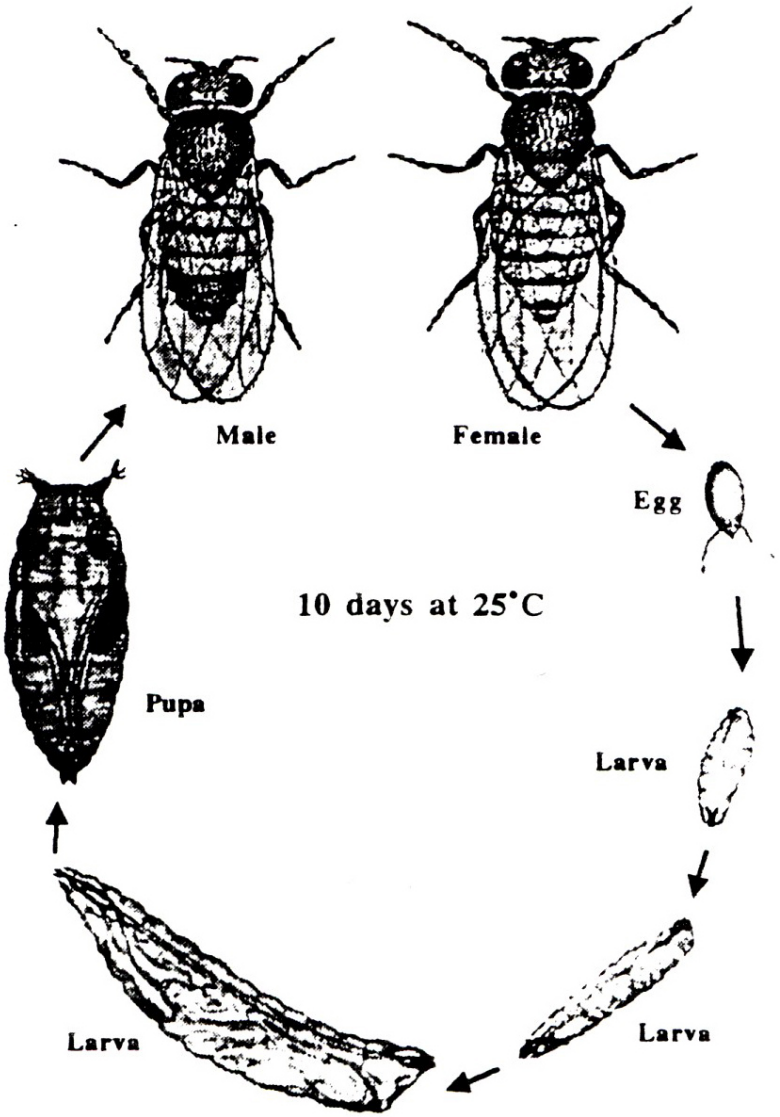
5) Fast mutant screening system

6) Easily characterized simple pathway compared to complex pathway of mammalian

7) Availability of stocks containing altered genes

8) Powerful molecular genetic techniques

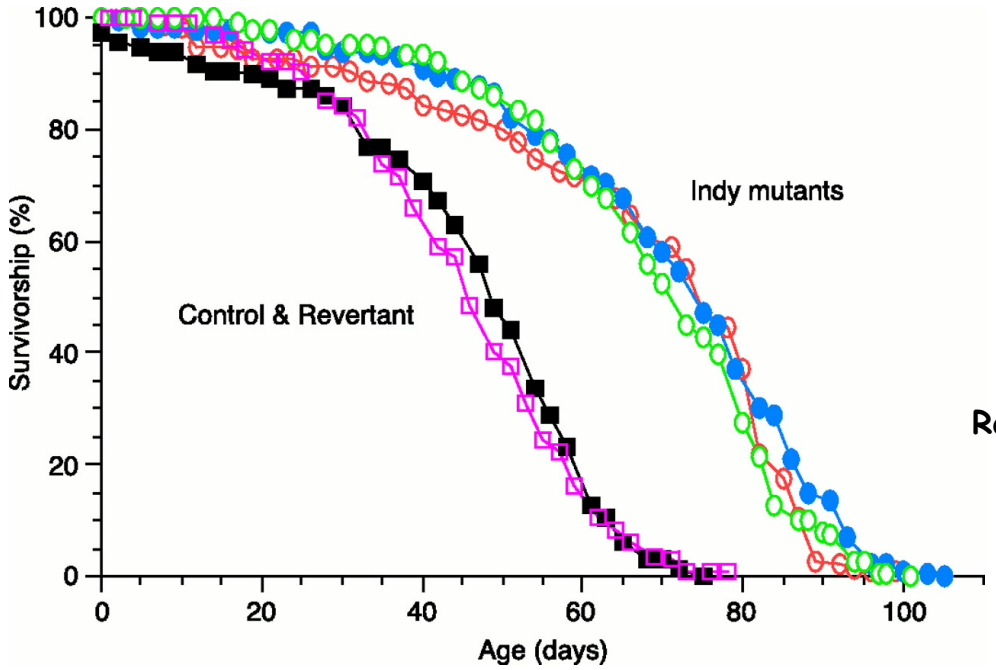
The Drosophila Life Cycle



Adult life-span : 2 ~ 3 months

Measuring aging in *Drosophila*

Survival of 200 adults on the cornmeal medium is followed (20 flies per vial) at 25^{0}C with enumeration and transfer of survivors to fresh vials every 2-3 days.



Rogina et al., Science 2000

1) Median life-span : the day in 50% of survivors
2) Maximal life-span : the day in last survivor
3) Mortality : the rate of flies dying per day

Non-Genetic factors to affect life-span in fly

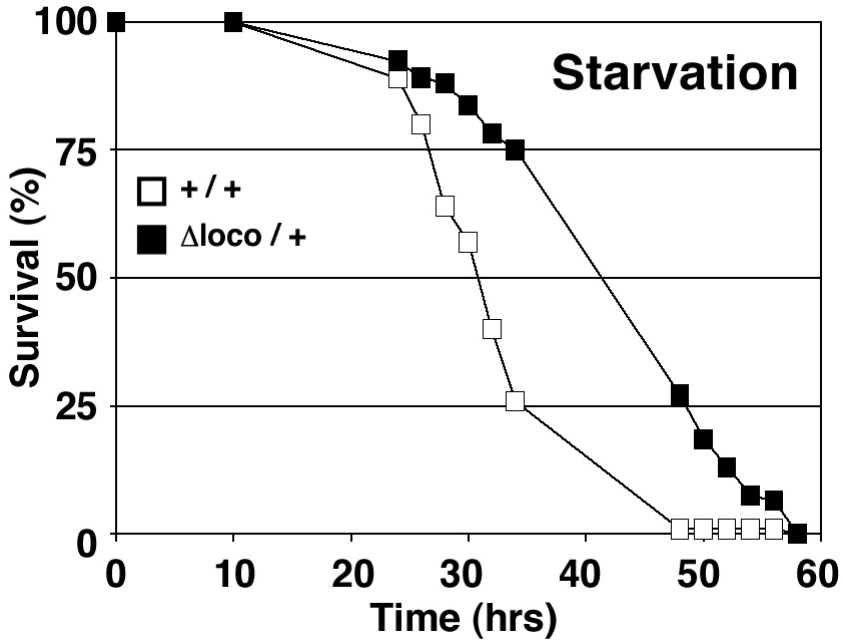
1) Stressor, 2) Temperature, 3) Reproductivity, 4) Diet

Stress response assay

Starvation test: a group of 100 flies (20 flies per vial) are maintained in the vials containing two filters wetted with 300 ul of water at 25C .

Oxidation test: adult flies, starved for initial 6 hrs, are maintained in the vials containing two filters wetted with 300 ul of 20 mM paraquat in 5% sucrose solution at 25C.

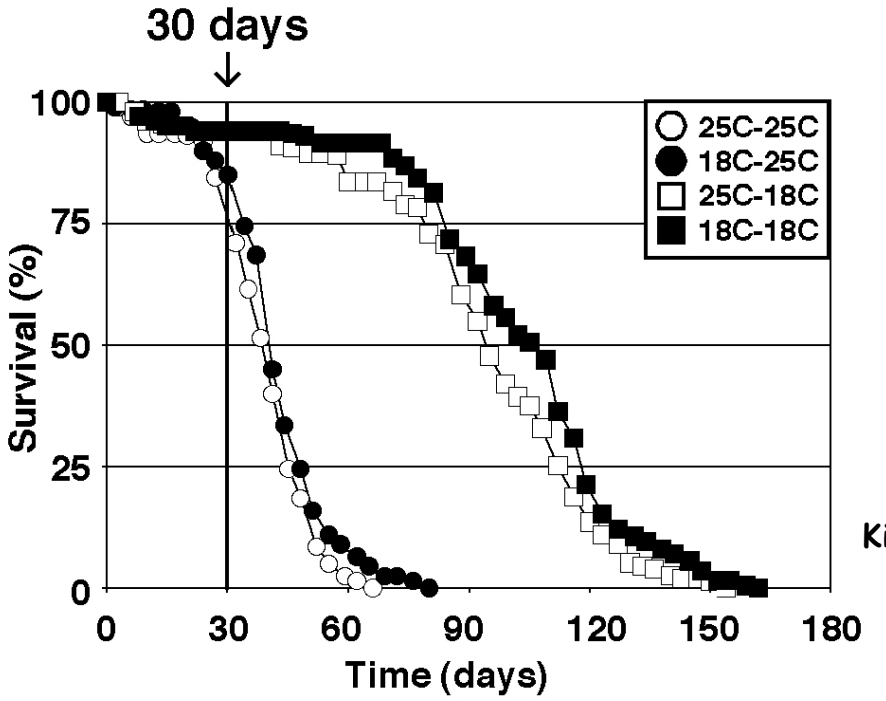
Heat test: adult flies are maintained in standard cornmeal vials at 37C with 30% humidity.



Lin et al., Aging Cell, 2011

Non-Genetic factors to affect life-span in fly

1) Stressor, 2) Temperature, 3) Reproductivity, 4) Diet

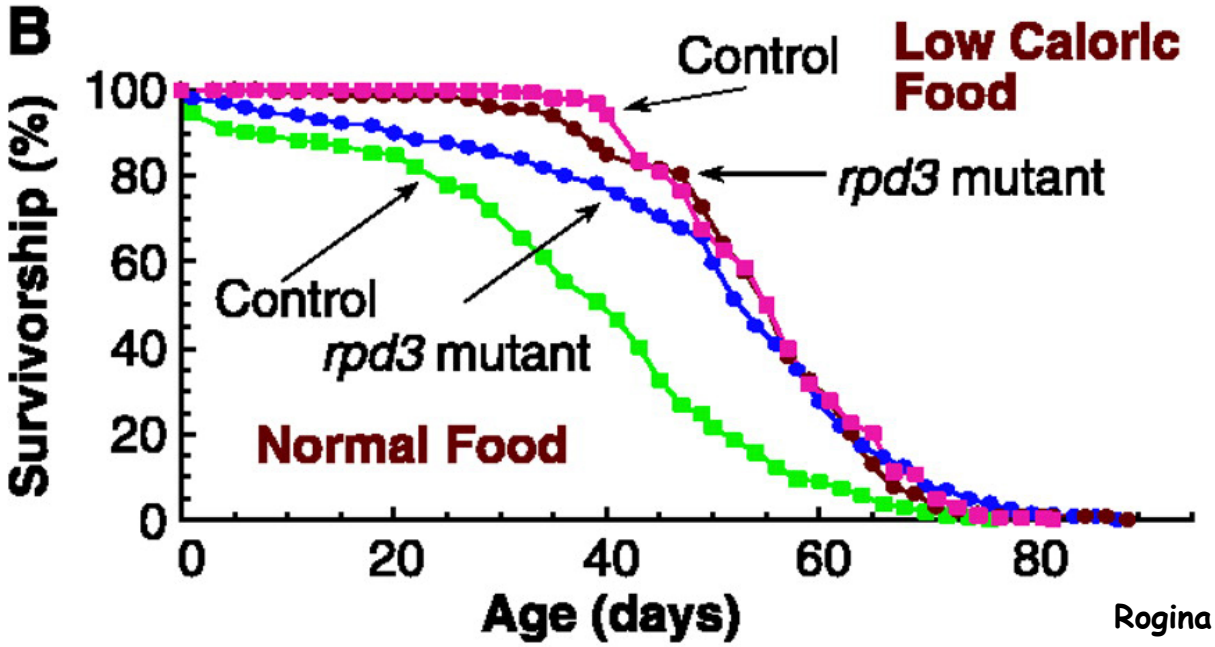


Kim et al., Exp. Gerontol., 2010

Lower temperature (18C) extends life-span of flies than higher temperature (25C).

Non-Genetic factors to affect life-span in fly

1) Stressor, 2) Temperature, 3) Reproductivity, 4) Diet



Rogina et al., Science 2002

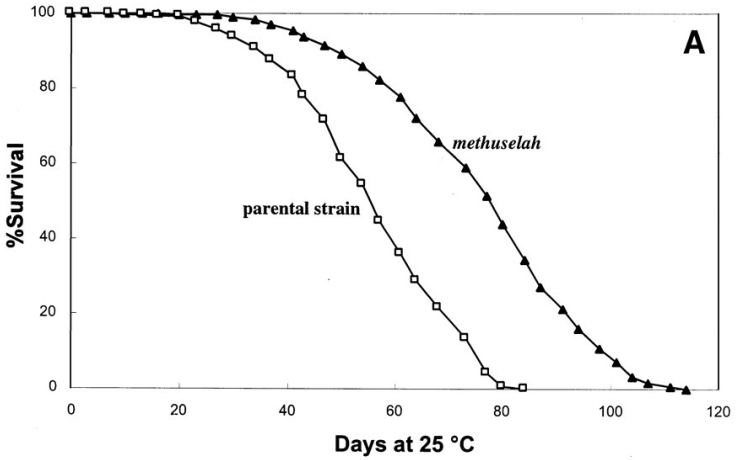
Genetic approaches to understanding aging

1) The random single-gene alternation approach
-mutagenesis (*mth*, *Indy*)
-ectopic expression

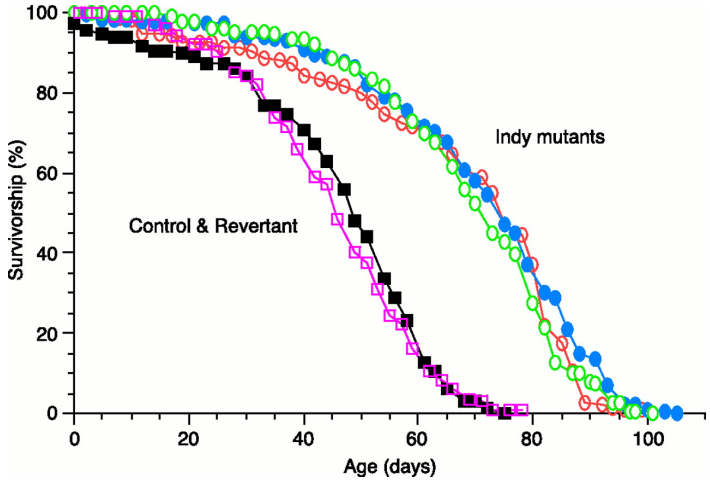
2) The candidate gene approach
-Antioxidants (catalase, SOD)
-Protective/Repair system (PCMT, MSRA)
-Insulin/IGF-like signaling (*InR*, *chico*)
-Chromatin structure (*rpd3*)

The random single-gene alternation approach

-Partial loss-of-function mutation in *methuselah* (*mth*), G-protein-coupled receptor, increases life span.



Lin et al., Science 1998



Rogina et al., Science 2000

-Partial loss-of-function mutation in *I'm not dead yet (Indy)*, sodium dicarboxylate cotransporter, increase life span.

Candidate gene alternation approach

1) The Antioxidant genes

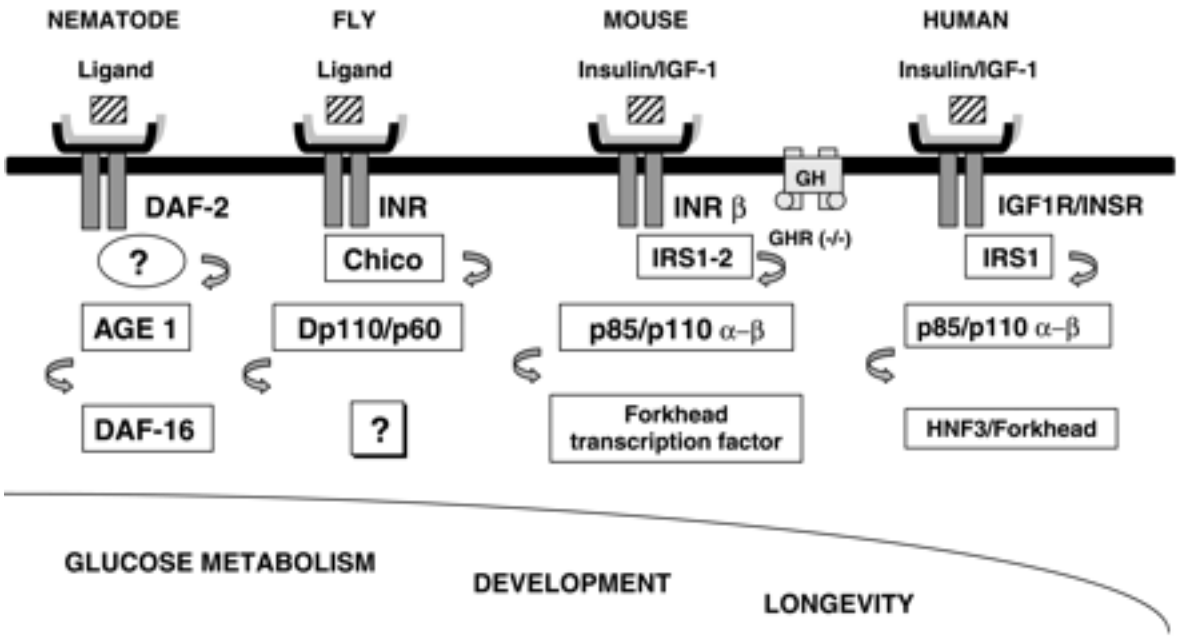
-The ability to slow the accumulation of oxidative damage extends life span.

-Increases in catalase and superoxide dismutase (SOD) show increases in life span.

Candidate gene alternation approach

2) The Insulin/IGF-like signaling genes

-Mutations in insulin receptor (*InR*) and insulin receptor substrate (*chico*) extend life span.
-The evolutionary conservation of longevity-determination pathway



Candidate gene alternation approach

3) The Protective/Repair system genes

-Increased protein repair systems extend life span.

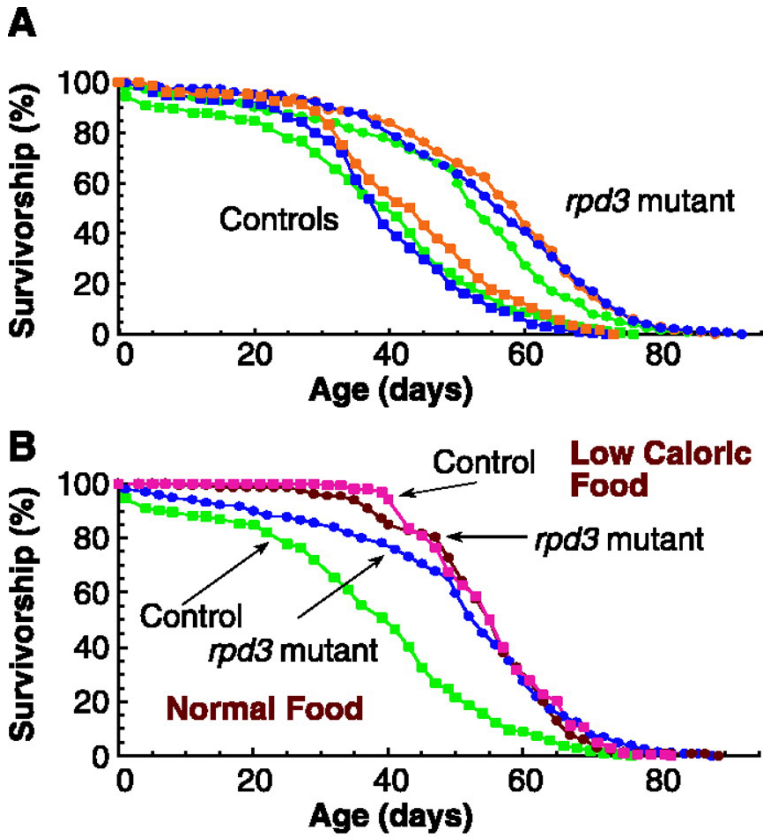
protein carboxy methytransferase (PCMT)
methionine sulfoxide reductase A (MSRA)

Candidate gene alternation approach

4) The Chromatin structure genes

-Phenylbutyrate (PBA), inhibitor of histone deacetylase also extends life span.

-A decrease in histone deacetylase (*rpd*^{3}) increases life span.



Rogina et al., Science 2002

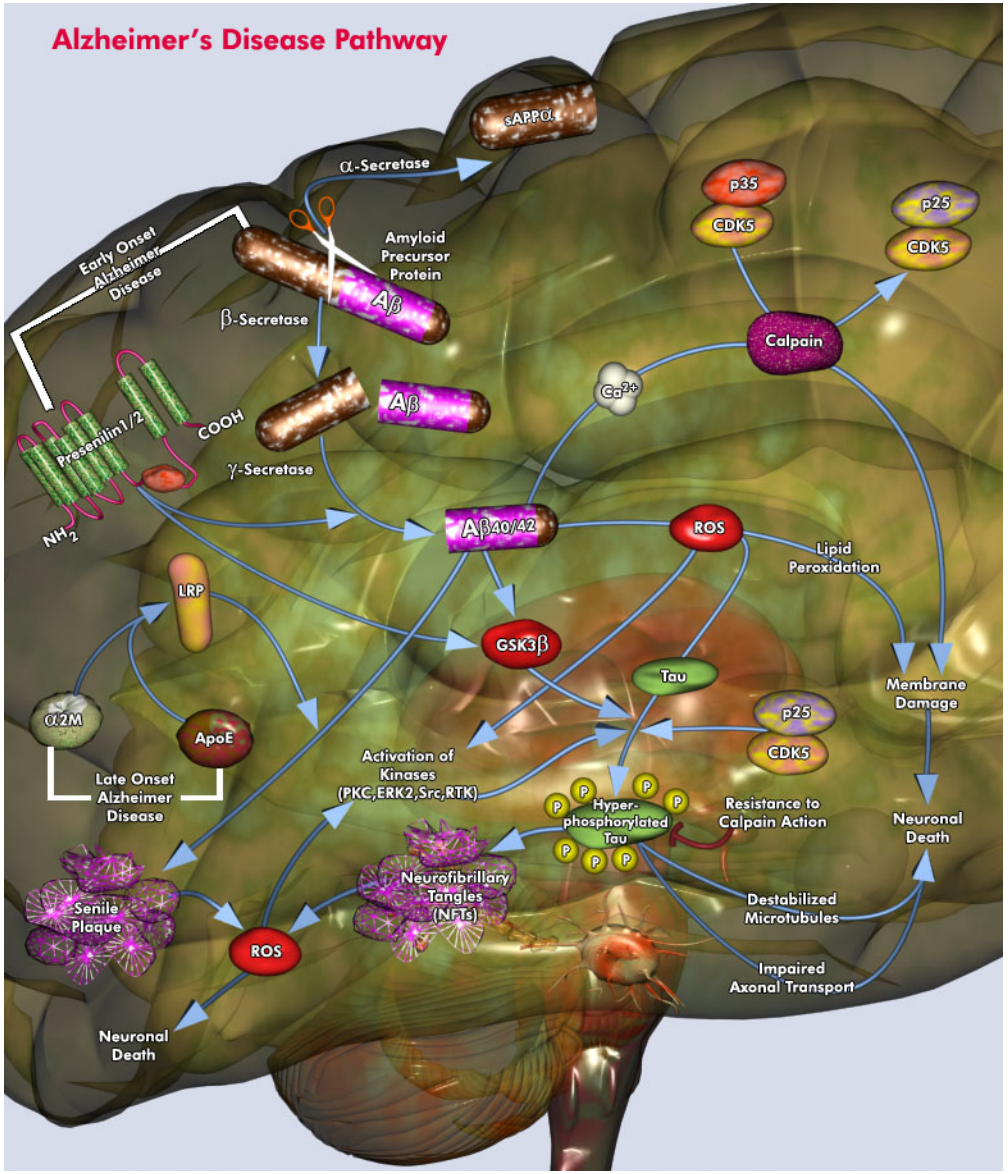
Alzheimer's Disease

AD (Alzheimer's Disease) is a neurodegenerative disorder leading to amnesia, cognitive impairment, and senile dementia.

The vast majority of cases of Alzheimer Disease are sporadic with no clear pattern of inheritance and a late age of onset (70s and 80s).

A small percent (5%) of cases, termed early-onset, arises at an unusually young age, as early as the third decade of life.

Mutations in **APP (Amyloid Precursor Protein)** and **PSn (Presenilin1/2)** are genetically associated with early-onset forms of familial Alzheimer Disease (FAD).



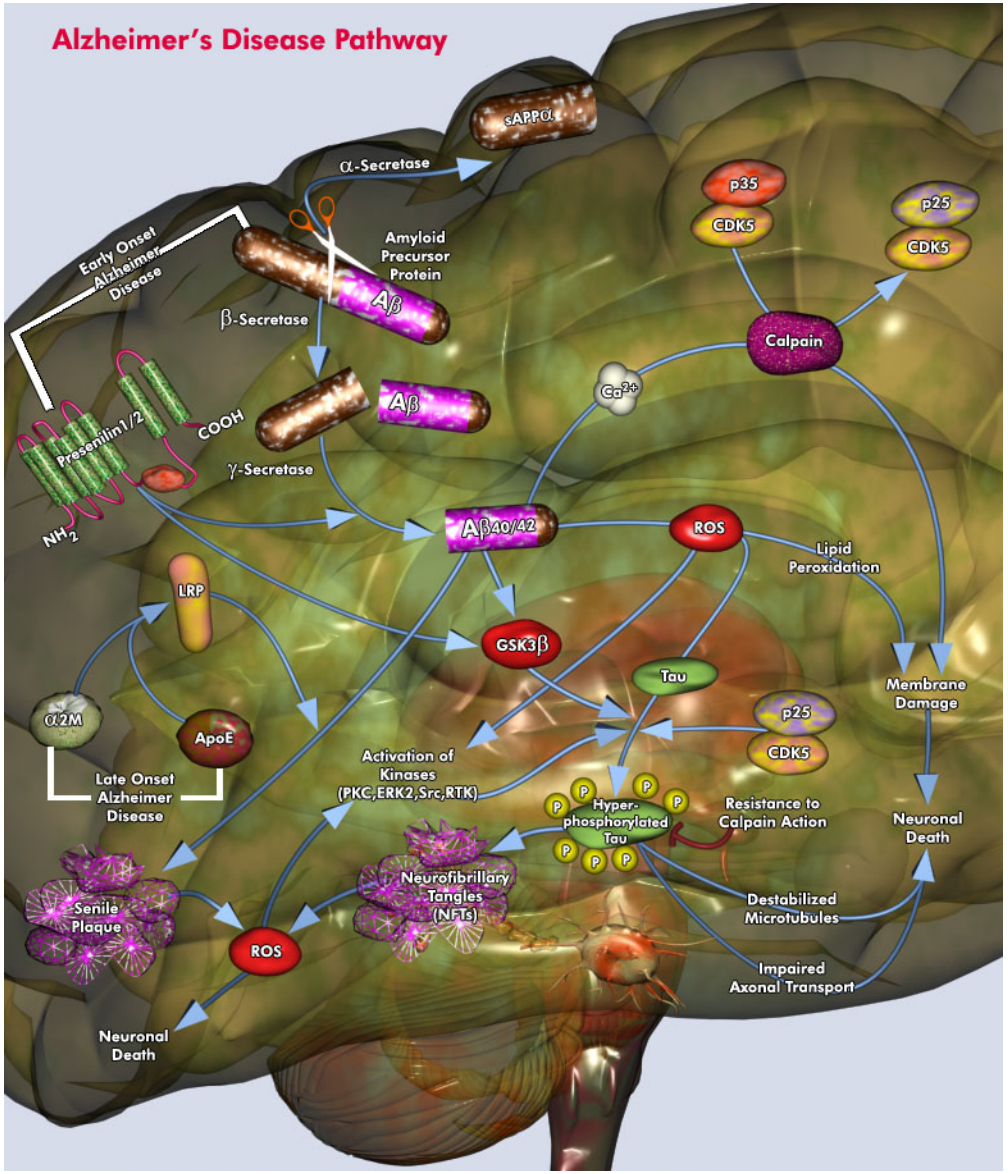
Two types of lesions extend progressively to neocortical brain areas during the course of Alzheimer Disease.

1) **SPs (Senile Plaques)** result from the extracellular aggregation of **Aβ (Amyloid Beta)** peptide, which is derived from APP (Amyloid Precursor Protein).

2) **NFTs (Neuro-Fibrillary Tangles)** are composed of intraneuronal bundles of PHF (Paired Helical Filaments). PHF result from the aggregation of pathologic **Tau** proteins, named PHF-Tau1.

SPs and NFTs generate **ROS (Reactive Oxygen Species**) such as hydrogen peroxide and hydroxyl radical that induce membrane lipid peroxidation, which results in impairment of the function of membrane glucose and glutamate transporters, altered mitochondrial function, and a deficit in ATP levels.

Cumulative ROS-induced membrane damage compromises membrane integrity and increases the permeability of several ions including calcium; resultant calcium influx is a crucial factor in neurodegeneration and leads to the **neuronal death**.



Alzheimer's Disease study in *Drosophila*

Drosophila as a powerful model to study age-related human neurodegenerative diseases including AD (Alzheimer's Disease)

1) Short generation time (~ 10 days) and short lifespan (~ 60–80 days)

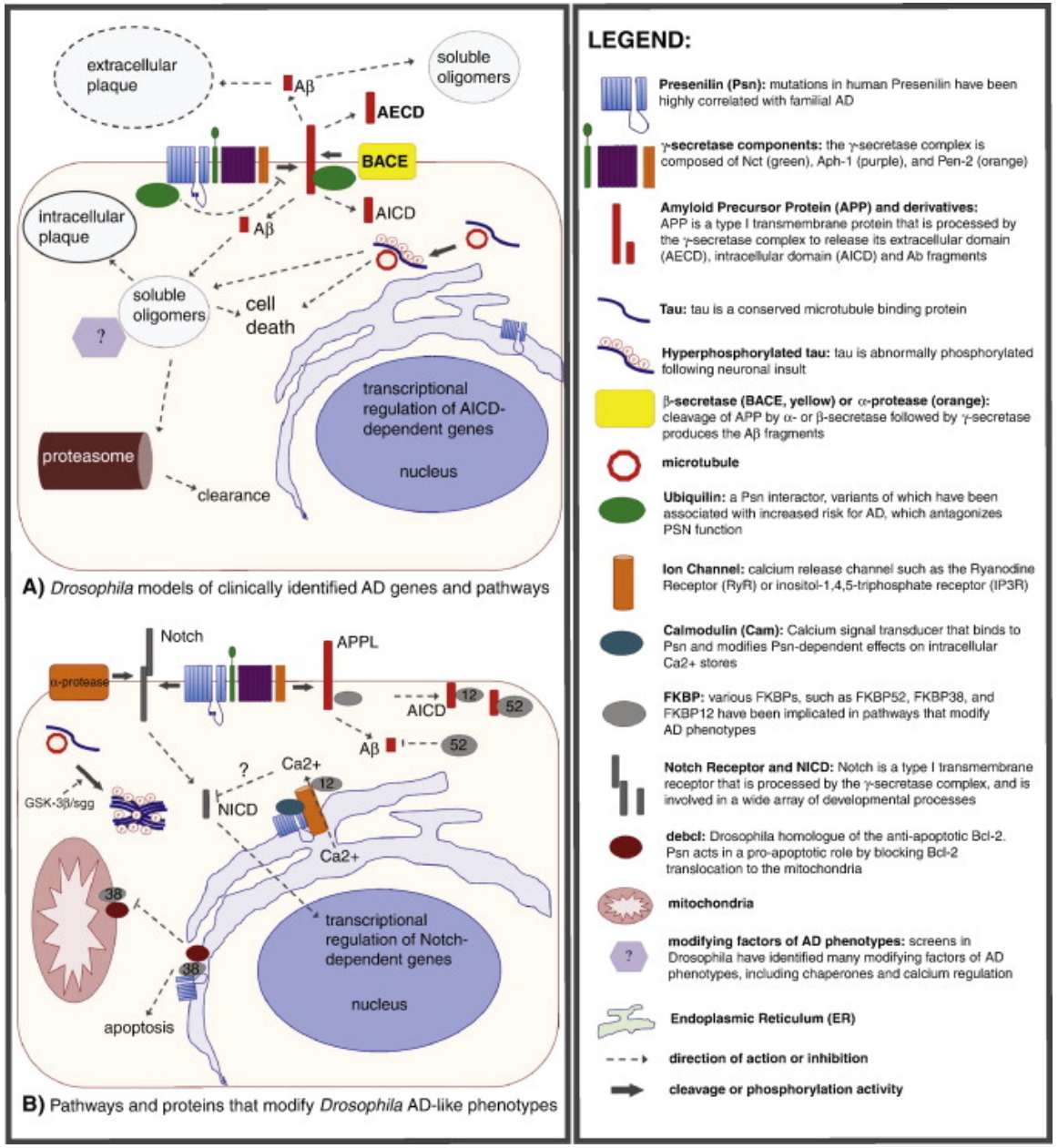
2) Conserved genes implicated in AD such as APP, PSn, and tau

3) Many tools to determine the effect of mutations on specific cell/tissue types, including neurons

4) Transgenic flies to express genes in a spatially and temporally restricted manner (UAS/Gal4 system)

5) Synaptic activity to be measured using electrophysiological and imaging techniques from both the neuromuscular junction and the adult central nervous system

6) Gene functions to be examined for their effects on various behaviors including locomotion, learning, and memory



Bonner & Boulianne, Exp. Gerontol. 2011

Alzheimer's Disease study in *Drosophila*

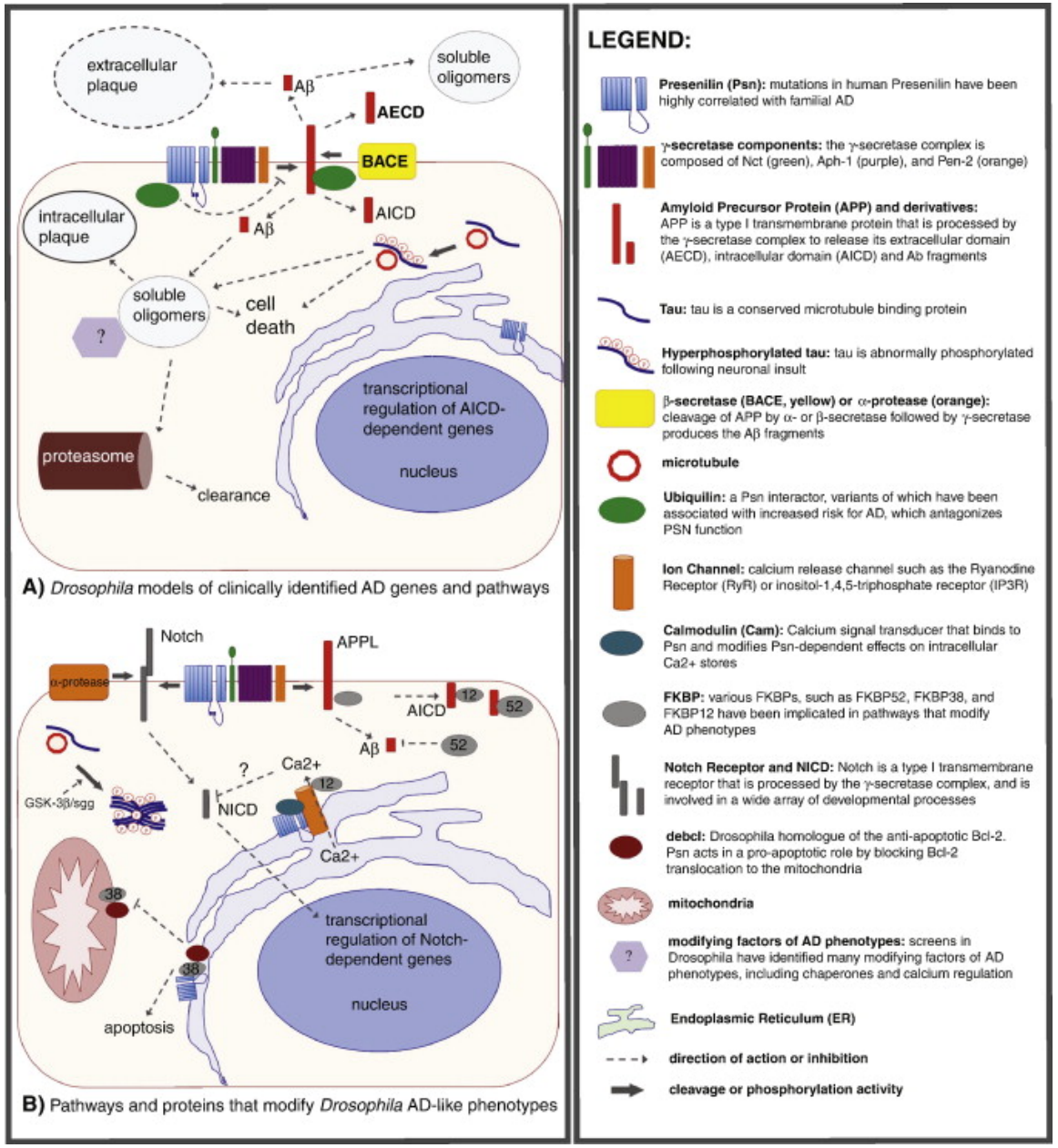
1. APP and Aβ transgenic models

1) Over-expression of dBACE (*Drosophila* BACE-like enzyme) and APPL (*Drosophila* APP homologue) results in the production of the Aβ peptide, which accumulates in neurotoxic aggregates and induces age-dependent, AD-like behavioural deficits and neurodegeneration.

2) Expression of Aβ peptide in the fly brain gives rise to clear amyloid deposits, age-dependent locomotor defects and neurodegeneration.

3) Over-expression of the PPIase FKBP52 reduces the toxicity of Aβ expression.

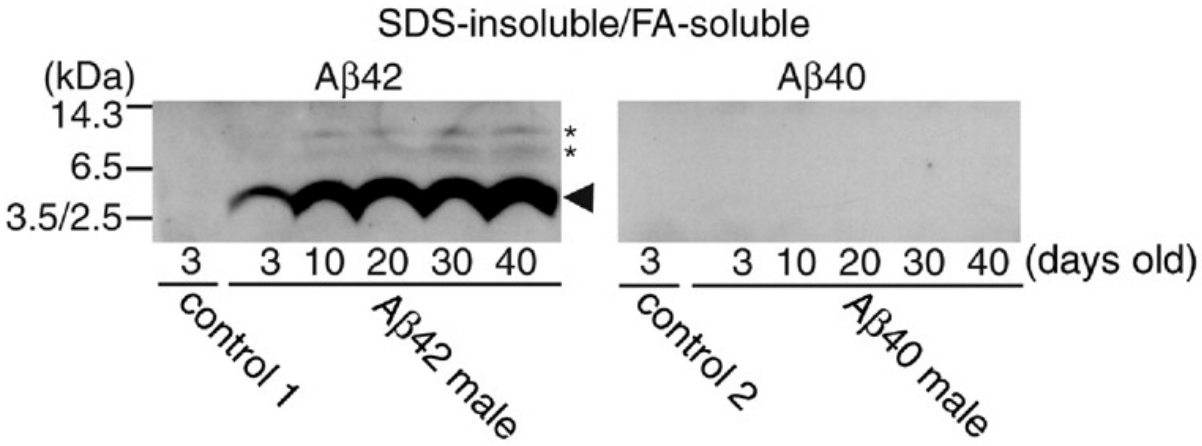
4) APP and Aβ-expressing flies recapitulates many important aspects of AD, including some phenotypes difficult to model in the mouse system, such as neuronal cell loss.

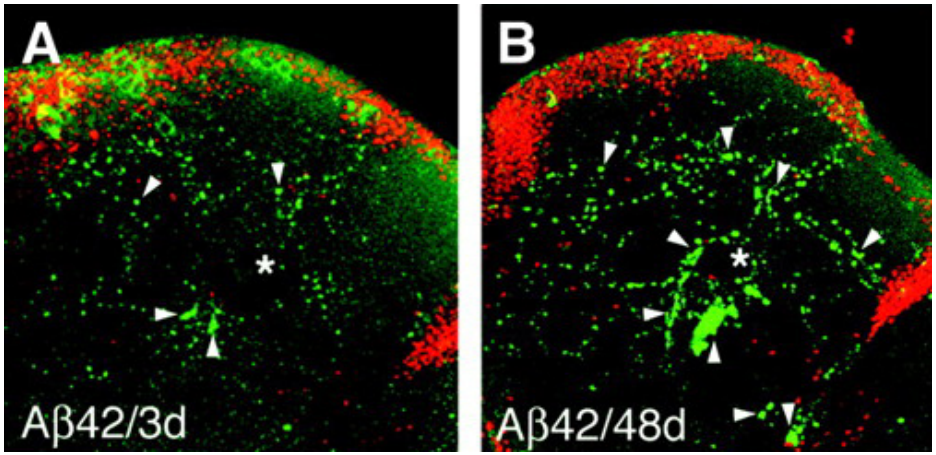


Bonner & Boulianne, Exp. Gerontol. 2011

Dissecting the pathological effects of human Aβ40 and Aβ42 in *Drosophila*: A potential model for Alzheimer's disease

Iijima K *et al.* PNAS 2004;101:6623-6628





Arrowhead: Aβ aggregates

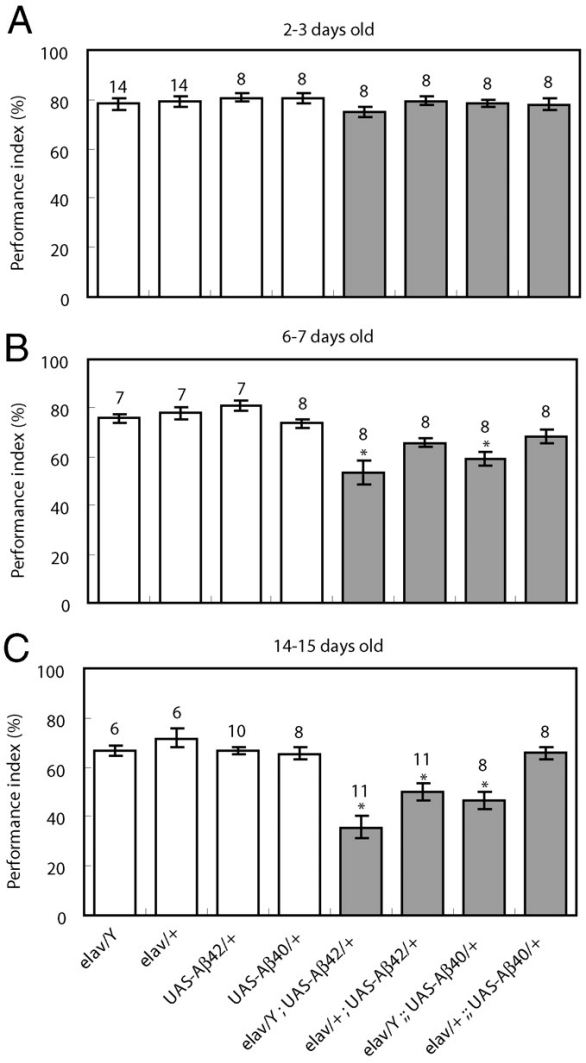
Age-dependent accumulation of Aβ peptides in fly heads as insoluble aggregates.

Dissecting the pathological effects of human Aβ40 and Aβ42 in *Drosophila*: A potential model for Alzheimer's disease

Iijima K *et al.* PNAS 2004;101:6623-6628

Pavlovian Olfactory Associative Learning

Flies are trained by exposure to electroshock paired with one odor {octanol [10^{-3} (vol/vol)] or methylcyclohexanol [10^{-3} (vol/vol)]} for 60 s and subsequent exposure to a second odor without electroshock for 60 s. Immediately after training, learning is measured by allowing flies to choose between the two odors for 120 s. The performance index is calculated by subtracting the number of flies making the incorrect choice from those making the correct one, dividing by the total number of flies, and multiplying by 100.



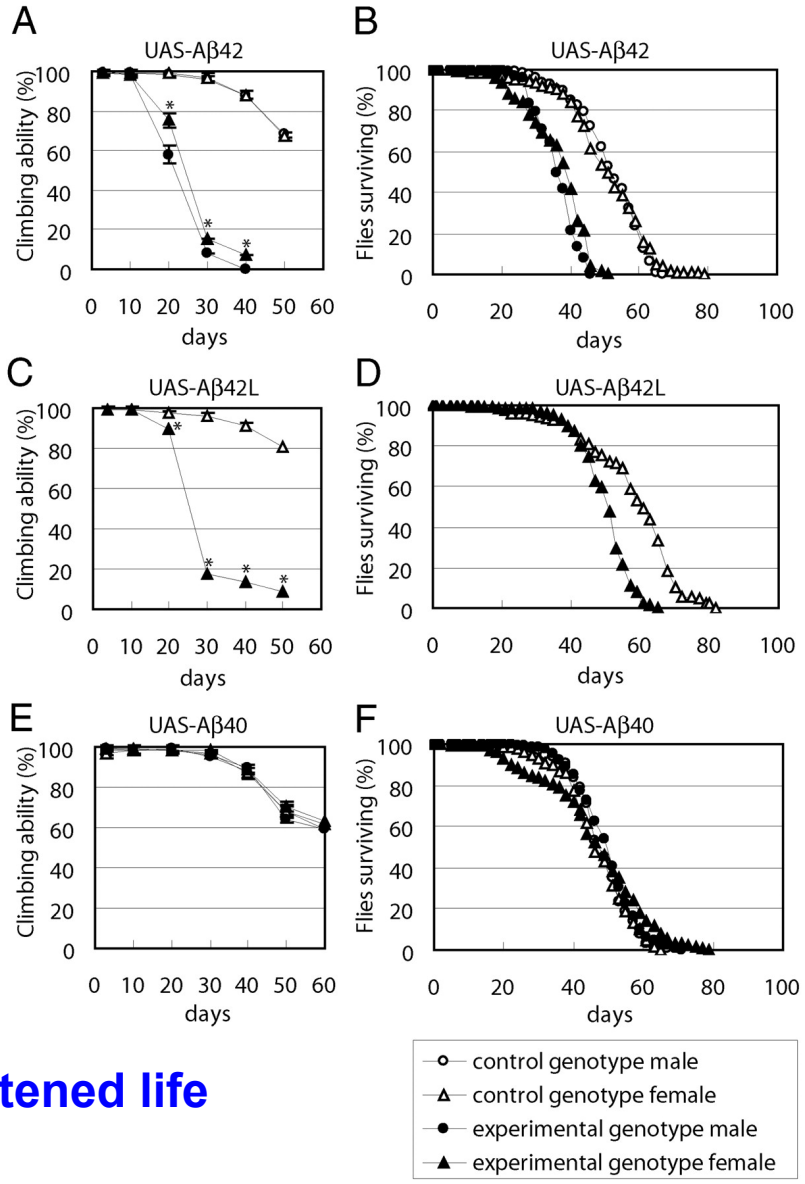
Progressive loss of learning ability in Aβ flies.

Dissecting the pathological effects of human Aβ40 and Aβ42 in *Drosophila*: A potential model for Alzheimer's disease

Iijima K *et al.* PNAS 2004;101:6623-6628

Climbing Assay

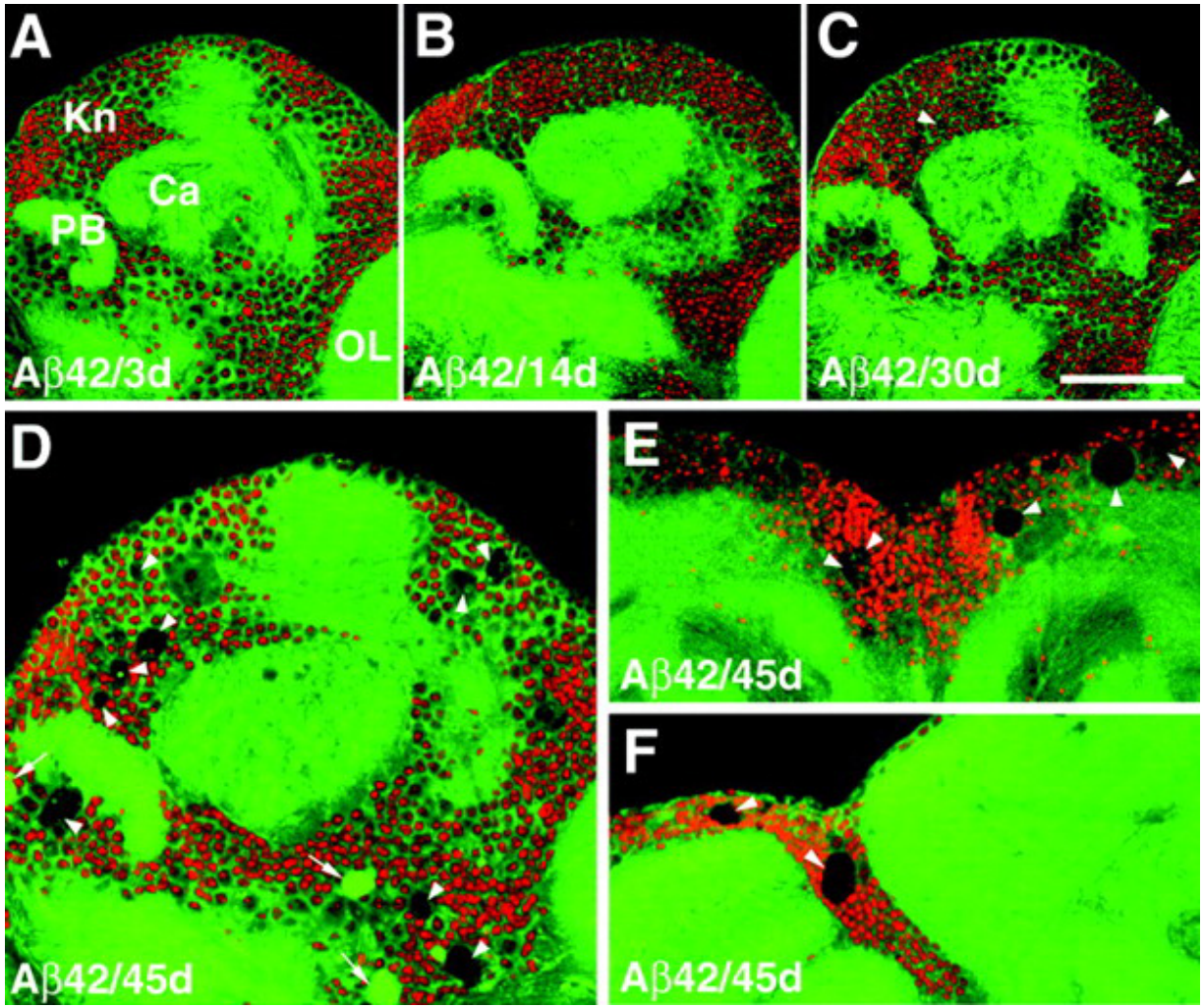
Twenty flies are placed in a plastic vial and gently tapped to the bottom. The number of flies at the top of the vial was counted after 18 s of climbing under red light (Kodak, GBX-2, Safelight Filter).



Progressive climbing disability and shortened life span in Aβ42 flies.

Dissecting the pathological effects of human Aβ40 and Aβ42 in *Drosophila*: A potential model for Alzheimer's disease

Iijima K *et al.* PNAS 2004;101:6623-6628



Arrowhead: Neuronal loss

Late-onset progressive neurodegeneration in Aβ42 brains.

Alzheimer's Disease study in *Drosophila*

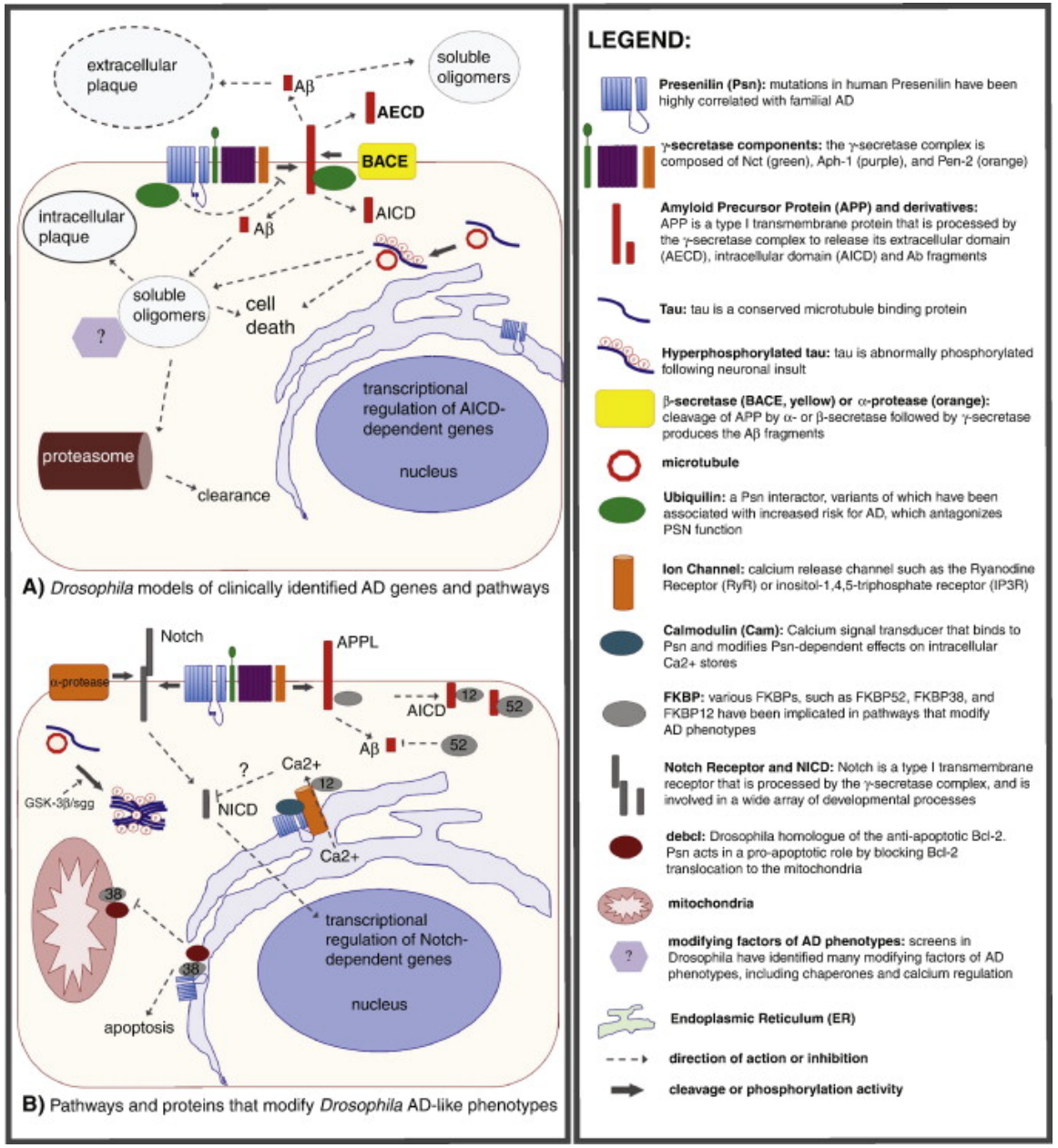
2. Presenilin and the γ-secretase complex in Drosophila AD models

1) Over-expression of PSn in *Drosophila* cholinergic neurons gives rise to intracellular calcium deficits as the earliest events in AD pathogenesis.

2) Mutations in conserved residues of *Drosophila* Psn have been used to model clinically heterogeneous human PS FAD mutations.

3) dUbqln (*Drosophila* Ubiquilin homologue) binds to PSn and antagonizes its function *in vivo*.

4) Inhibitors against BACE or γ-secretase suppress age-dependent neuro-degeneration that results from Aβ peptide aggregates.



Bonner & Boulianne, Exp. Gerontol. 2011

Alzheimer's Disease study in *Drosophila*

3. Tau-based models of AD

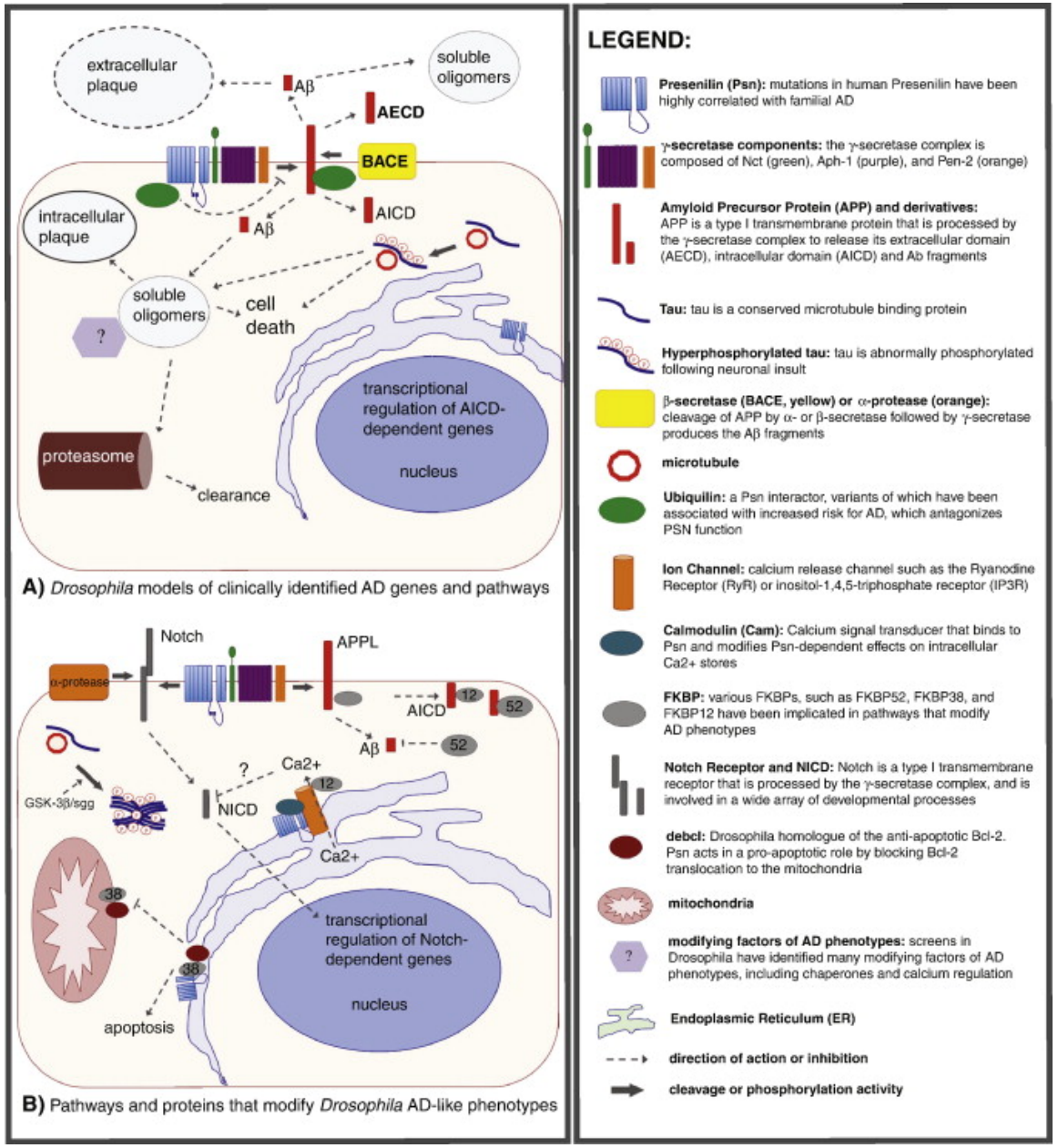
1) Expression of human mutant tau transgene shortens the lifespan of flies and exhibits age-dependent neurodegeneration and vacuolization associated with increased staining for NFT-specific epitopes.

2) Expression of human wild-type tau in combination with shaggy, the *Drosophila* homologue of the kinase GSK-3β, is associated with cell loss, vacuolization and abnormal nuclear lamin accumulation suggestive of apoptosis.

4. Genetic screens to identify modifiers of AD-like degenerative phenotypes

1) Modifiers of PSn-dependent phenotypes, which enhance or suppress wing and bristle phenotypes due to over-expression of wild-type Psn.

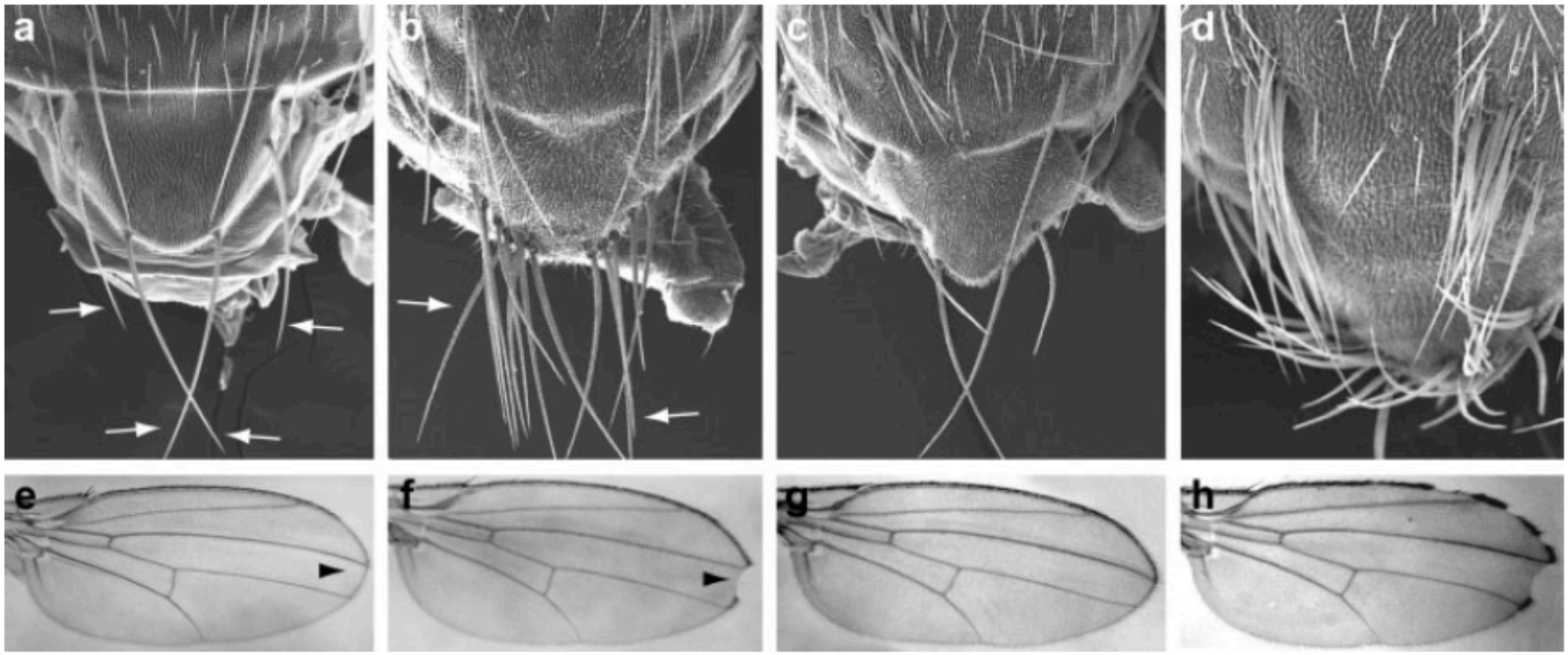
2) Candidate screening of pharmacological compounds to treat AD: γ-secretase inhibitors in Drosophila cell culture.



Bonner & Boulianne, Exp. Gerontol. 2011

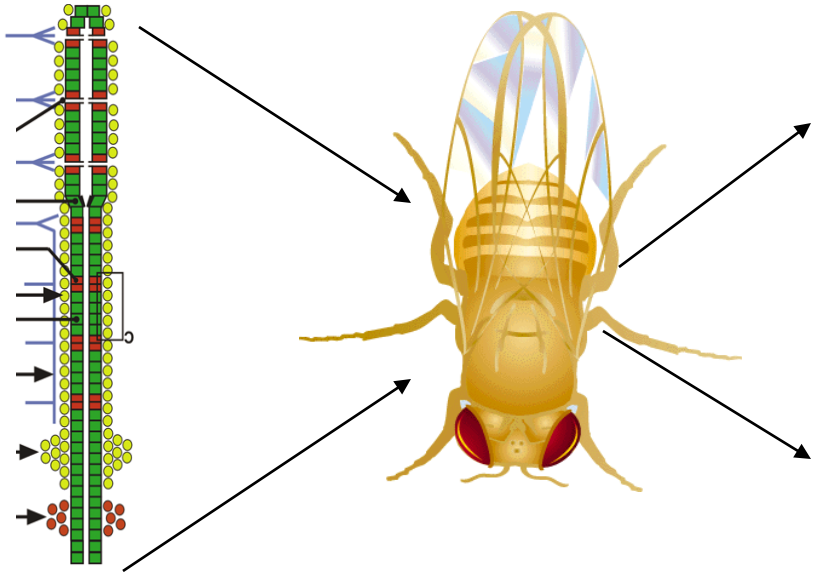
Identifying genes that interact with *Drosophila* presenilin and amyloid precursor protein

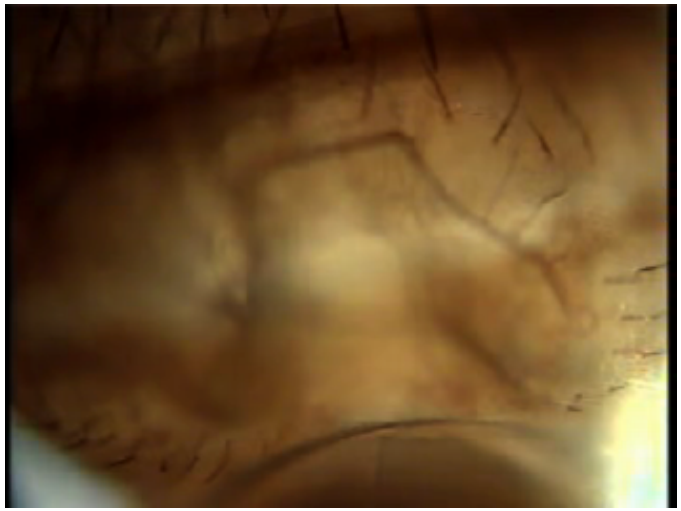
Van de Hoef *et al.* Genesis 2008;47:246-260



Wild-type **PSn over-expression** **Suppressor** **Enhancer**

Heart Study in the Fruitfly





Drosophila is a powerful model organism for the analysis of human disease genes

1) Online Mendelian Inheritance in Man (OMIM)

2) 74% of 1682 human disease gene has homologs in *Drosophila*.

3) A third of these genes (~ 500) are functionally equivalent between flies and humans.

4) Neurological disorders, developmental defects, metabolic/storage disorders, cancer, cardiovascular disease, the visual, auditory, and immune systems disorders.

Congenital Heart Disease (CHD) and Cardiac Arrest

1) Cardiac development (e.g., transcription factors)
   cardiac muscle contraction (e.g., cytoskeletal proteins)
   conduction of electrical signals (e.g., ion channels)
   hypertension (e.g., peptide hormone signaling)
   formation of veins and arteries (e.g., receptor tyrosine kinase signaling)

2) Cardiovascular disease is the leading cause of death (~ 950,000/year) in the United States.

3) Genes controlling early stages of heart development have been highly conserved in vertebrates and invertebrates

4) The *Drosophila* homologs of human cardiac disease and *Drosophila* genes to interact with these homologs

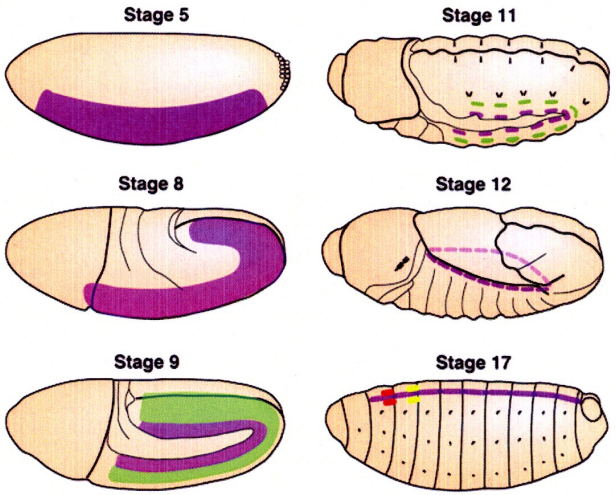
Cardiac disease genes and *Drosophila* homologs

Table 1
Cardiac disease genes within nondevelopmental disease categories that have *Drosophila* counterparts

Disease category	Disease	OMIM #	e-Value	Fly genes	Alleles	Protein function
Cardiomyopathy	Cardiomyopathy, familial hypertrophic, 1	160760	e-300	*mhc*	70	Cardiac myosin heavy chain-β
	" "	" "	e-300	*zip*	20	" "
	Cardiomyopathy, hypertrophic, midventicular chamber type (3)	160790	2e-34	*Mlc-c*	1	Essential light chain of myosin
	Cardiomyopathy, hypertrophic, midleft ventricular chamber type (3)	160781	3e-42	*sqh*	15	Myosin ATPase
	Cardiomyopathy, familial hypertrophic, 9	188840	e-300	*bt*	39	Titin: myosin light chain kinase
	Cardiomyopathy, familial hypertrophic,4	600958	e-70	*CG18242*	pl(3)j1D7	Cardiac myosin binding protein C
	Cardiomyopathy, familial hypertrophic, 3	191010	2e-63	*Tm1*	1	Tropomyocin
	Cardiomyopathy, familial hypertrophic, 2	191045	5e-12	*up*	14	Troponin complex
	Cardiomyopathy, familial hypertrophic, 3	191044	e-10	*wupA*	24	Troponin I, cardiac form
	Cardiomyopathy, idiopathic dilated	102540	e-300	*Act57B, 79B, 87E*	1 each	Smooth muscle actin
	" "	" "	e-300	*Act42A*	pEP(2)2096	" "
	" "	" "	e-300	*Act5C*	6	" "
	" "	" "	e-300	*Act88F*	80	" "
	Becker muscular dystrophy, cardiomyopathy, dilated, X-linked, Duchenne muscular dystrophy	310200	e-300	*Dys*	1	Dystrophin
	Cardiomyopathy (1), myopathy, desminopathic	125660	3e-37	*Lam*	8	Desmin
	Barth syndrome	302060	3e-61	*tafazzin*	3	Phospholipid and glycerol acyltransferase?
Conduction	Jervell and Lange–Nielsen syndrome	192500	e-64	*CG12215*	pEP(2)2074	Potassium channel
	Long QT syndrome-2	152427	e-300	*sei*	11	Potassium channel
	Long QT syndrome-3	600163	e-300	*para*	42	Sodium channel

	idiopathic dilated					
	" "	" "	e-300	*Act42A*	pEP(2)2096	" "
	" "	" "	e-300	*Act5C*	6	" "
	" "	" "	e-300	*Act88F*	80	" "
	Becker muscular dystrophy, cardiomyopathy, dilated, X-linked, Duchenne muscular dystrophy	310200	e-300	*Dys*	1	Dystrophin
	Cardiomyopathy (1), myopathy, desminopathic	125660	3e-37	*Lam*	8	Desmin
	Barth syndrome	302060	3e-61	*tafazzin*	3	Phospholipid and glycerol acyltransferase?
Conduction	Jervell and Lange–Nielsen syndrome	192500	e-64	*CG12215*	pEP(2)2074	Potassium channel
	Long QT syndrome-2	152427	e-300	*sei*	11	Potassium channel
	Long QT syndrome-3	600163	e-300	*para*	42	Sodium channel
	Artial septal defect with atrioventricular conduction defects	600584	2e-26	*vnd*	36	Transcription factor
	Stress-induced polymorphic ventricular tachycardia	604772	e-300	*Rya-r44F*	5	Ryanodine receptor (release of internal Ca++ stores)
	Arrhythmogenic right ventricular dysplasia	600996	e-300	*Rya-r44F*	" "	" "
Hypertension	Hypertension, salt-resistant	108962	3e-43	*CG3216*	1	Natriuretic peptide receptor C (receptor guanylate cyclase)
	Hypertension, essential, susceptability	139130	e-164	*Gbeta13F*	pEP(x)1071	Guanine nucleotide-binding protein, β-3
	Hypertension, essential	106165	4e-24	*AR-2*	1	Angiotensin/allatostatin receptor
	Hypertension, essential	145505	e-38	*AcCoAS*	pl(3)00217	Acetyl CoA synthetase
	Preeclampsia/eclampsia	199900	e-300	*Nos*	1	Nitric oxide synthetase
	Hypertension, pregnancy induced	163729	e-300	*Nos*	1	Nitric oxide synthetase
	Hypertension due to apparent mineralocorticoid excess	218030	8e-15	*CG8888*	1	Hydroxysteroid dehydrogenase
Atherosclerosis	Atherosclerosis, susceptibility	131210	6e-50	*fw*	25	Cell adhesion: selectin
	Myocardial infarction	106180	e-153	*Ance*	5	Angiotensin I converting enzyme
	Coronary artery disease	152200	4e-35	*CG10663*	1	Serine endopeptidase
Vascular defects	Venous malformations	600221	6e-63	*htl*	21	Receptor tyrosine kinase
	Cavernous angiomatous malformations	116860	7e-18	*CG11848*	1	Ras-interacting protein

Formation of Fly Heart (dorsal vessel)



Cripps and Olson, Dev. Biol. 2002

1) Activation of *tin* expression: a central event in the control of heart development mesoderm, dorsal mesoderm, heart precursors, cardial and pericardial cells

2) Tinman (homeodomain transcription factor): activator or repressor

3) In the absence of *tin* function, no heart precursors are generated.

4) Tinman binds to the consensus sequence 5' -TYAAGTG-3'

5) Nkx2.5 (vertebrate homolog of Tinman) can rescue *tin* mutant of *Drosophila*, when a unique N-terminal domain of Tinman is transferred to Nkx2.5.

Structure of Fly Heart

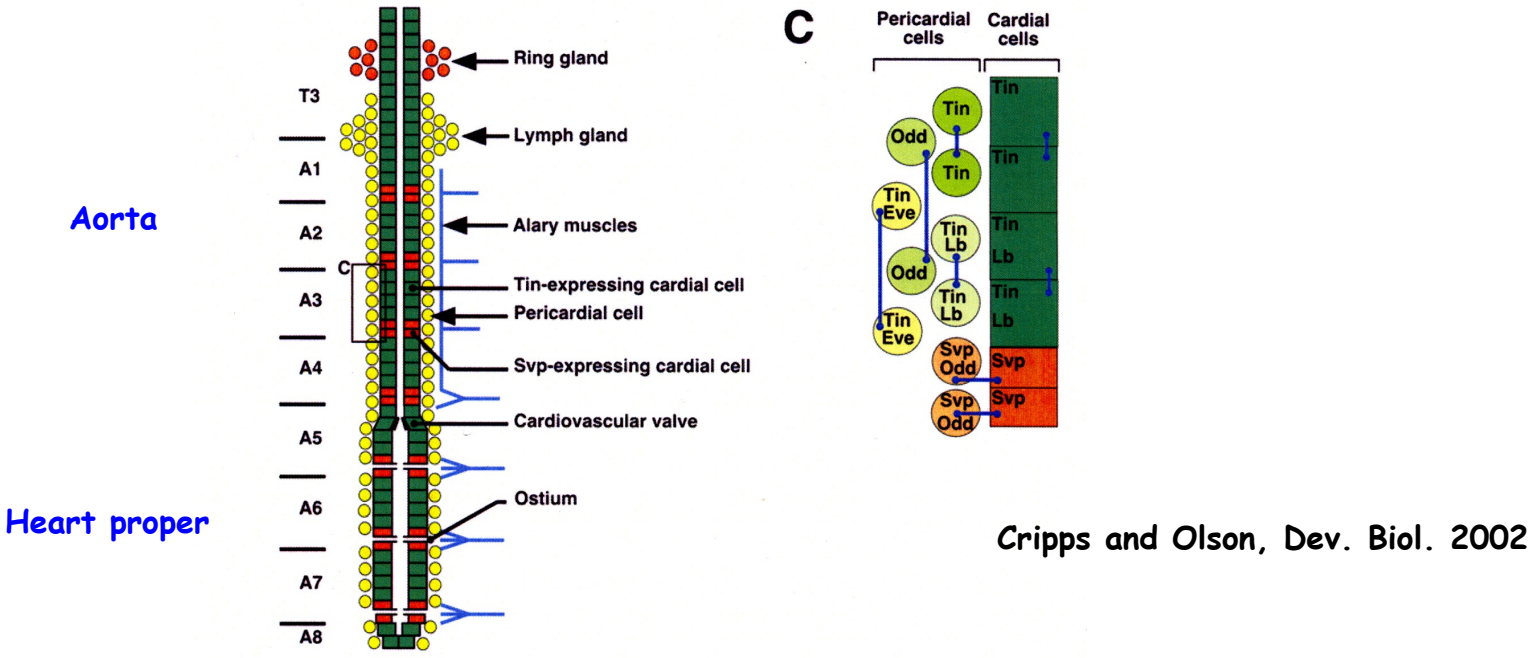
1) Blood (hemolymph) is pumped anteriorly toward the brain and then percolates posteriorly through the body cavity in an open circulatory system until it reenters the heart through inflow tracts termed ostia.

2) Cardial cell: contractile

3) Pericardial cell: macrophages, blood filtration

4) Lymph glands: blood-forming organ

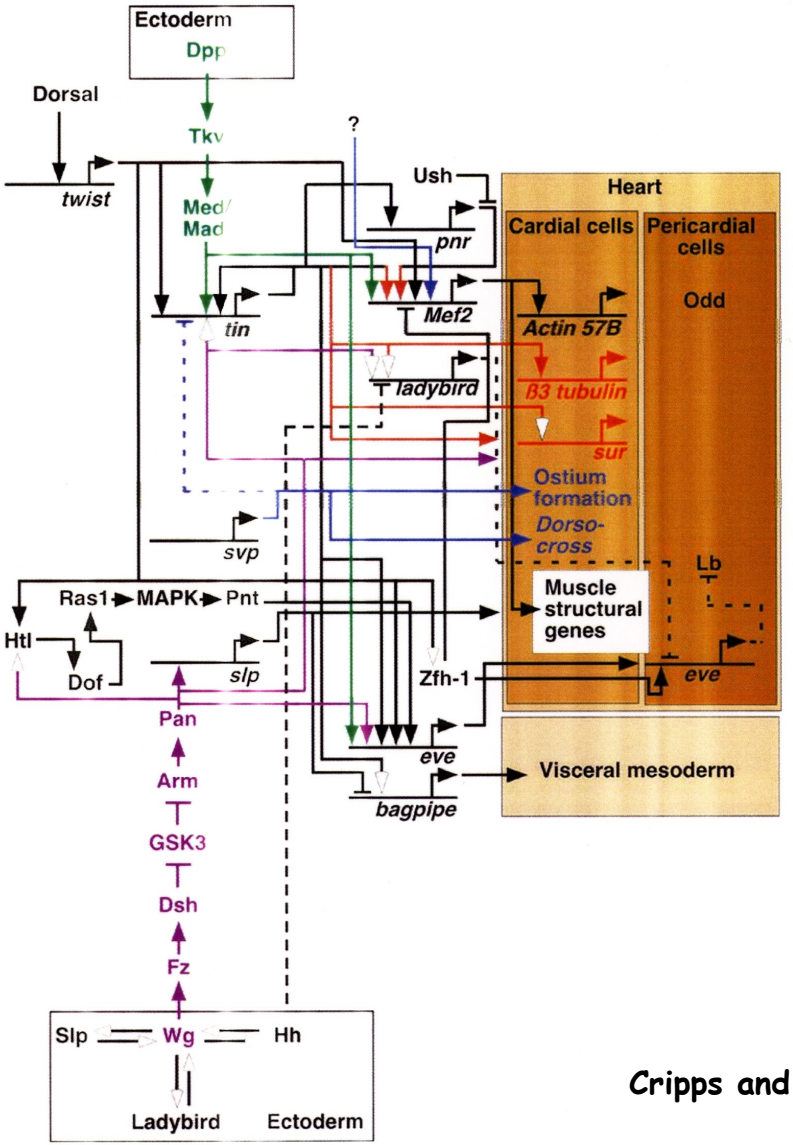
5) Ring gland: endocrine organ



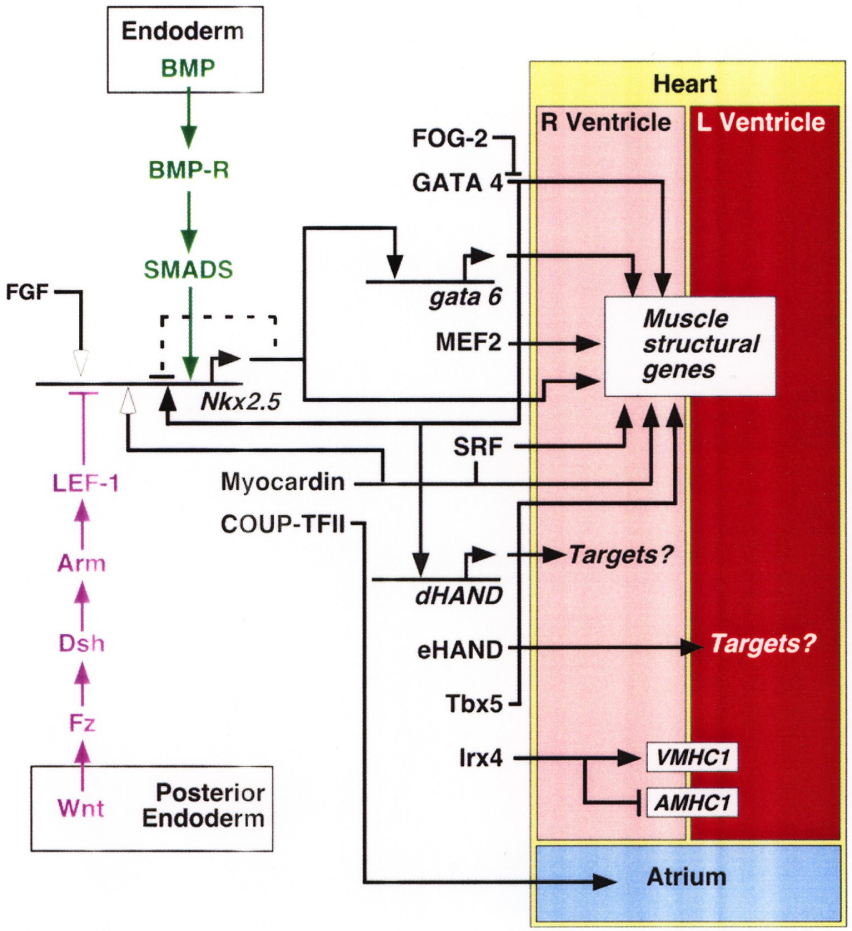
Cripps and Olson, Dev. Biol. 2002

Transcriptional Network for Cardiogenesis

In *Drosophila*

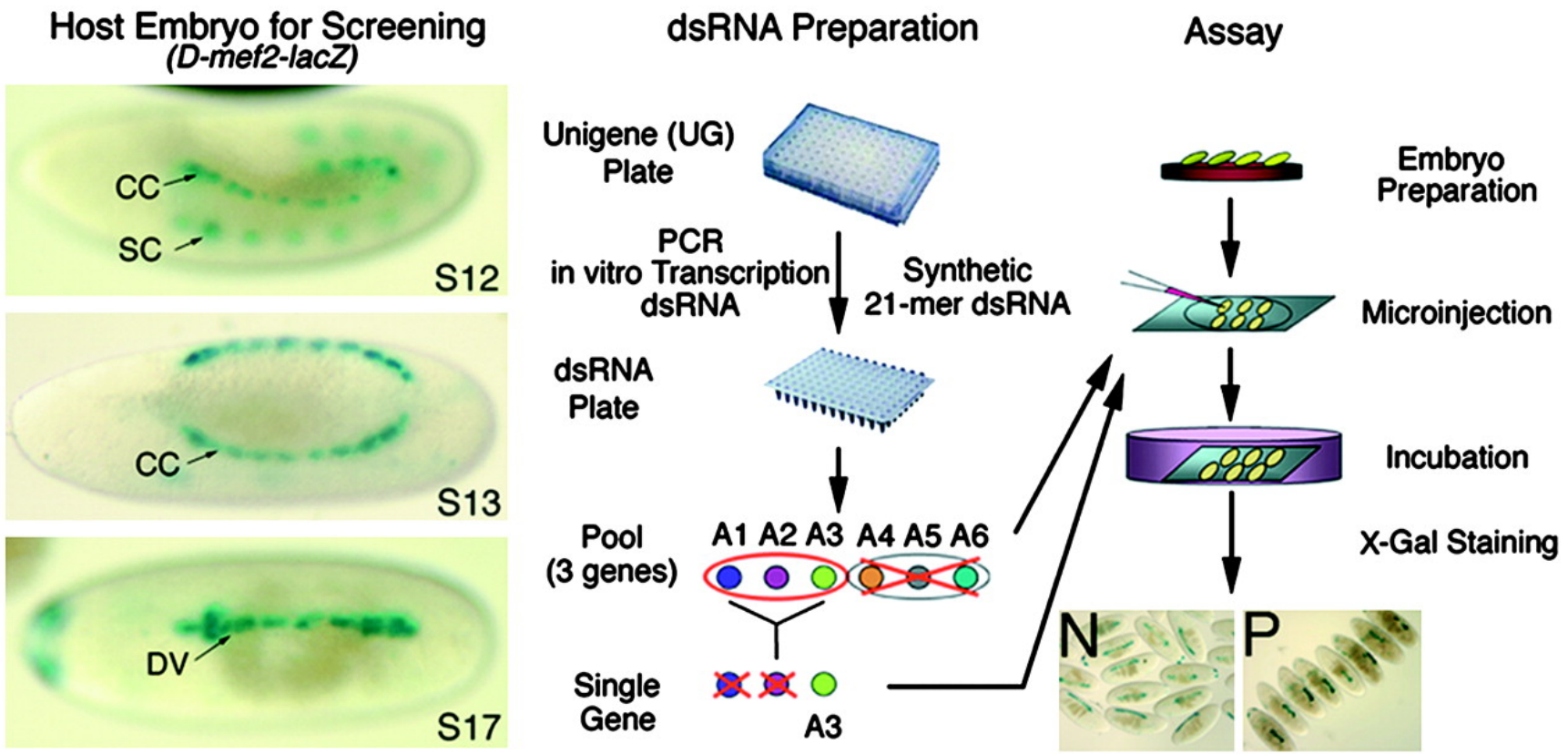


In Vertebrate



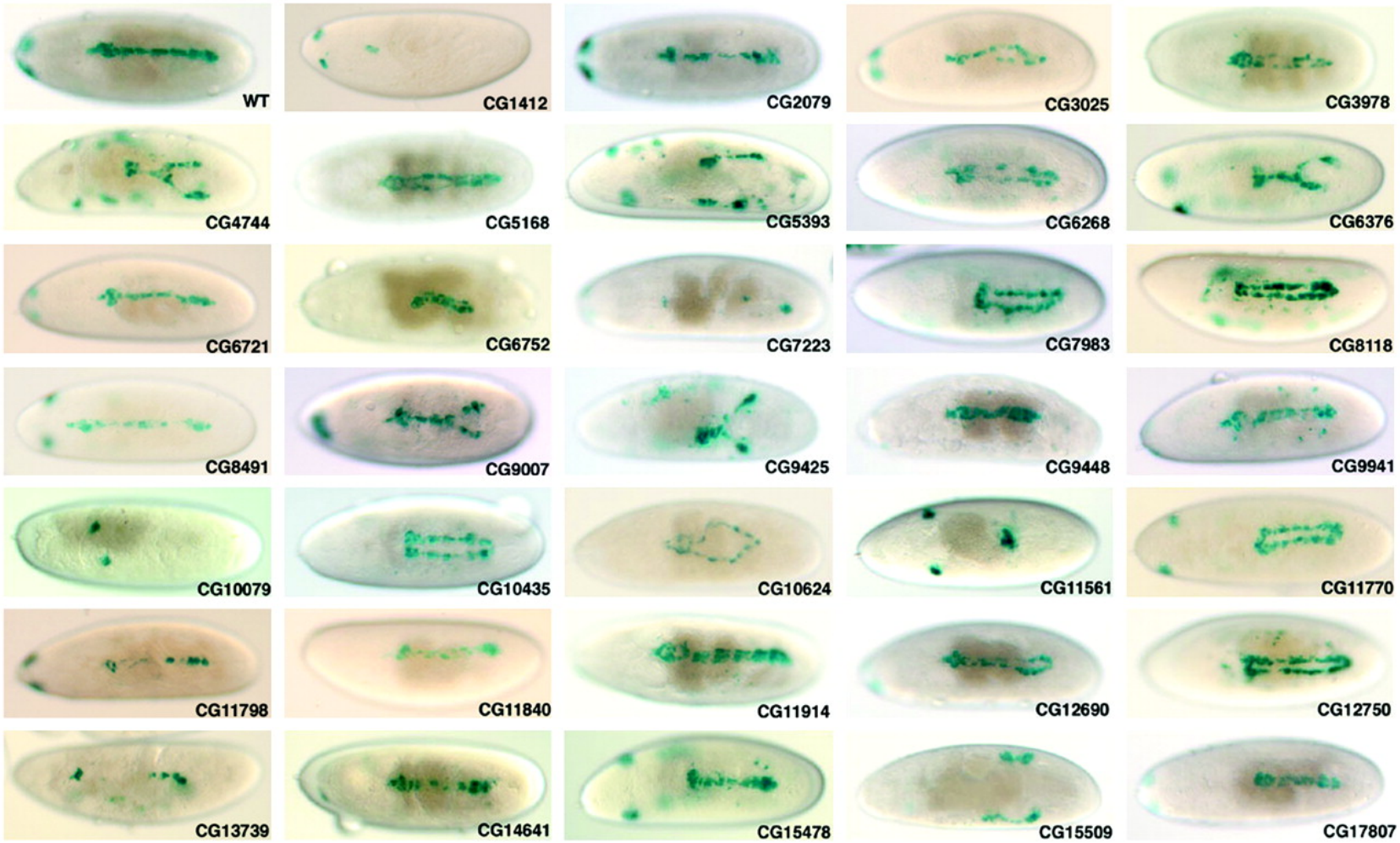
Cripps and Olson, Dev. Biol. 2002

Functional Genomic screen for Cardiogenic genes



Kim et al., PNAS 2004

Various heart phenotypes generated by RNAi



Kim et al., PNAS 2004

Insulin regulation of heart function in aging fruit flies

Robert J Wessells[1], Erin Fitzgerald[1], James R Cypser[2], Marc Tatar[2] & Rolf Bodmer[1]

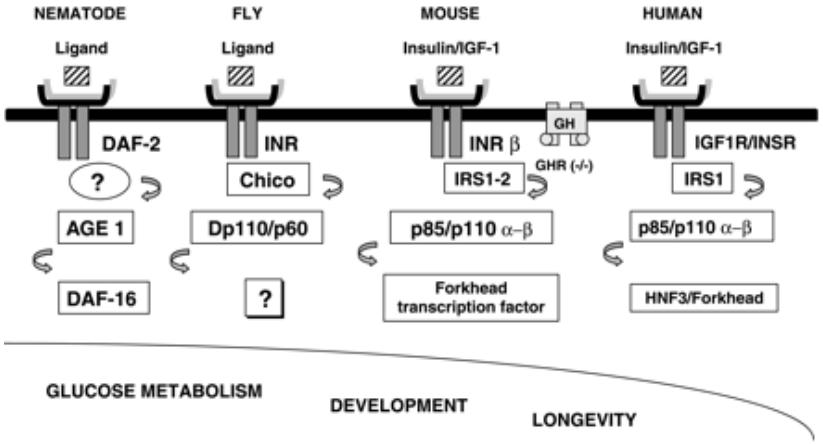
Insulin-IGF receptor (InR) signaling has a conserved role in regulating lifespan, but little is known about the genetic control of declining organ function. Here, we describe progressive changes of heart function in aging fruit flies: from one to seven weeks of a fly's age, the resting heart rate decreases and the rate of stress-induced heart failure increases. These age-related changes are minimized or absent in long-lived flies when systemic levels of insulin-like peptides are reduced and by mutations of the only receptor, InR, or its substrate, chico. Moreover, interfering with InR signaling exclusively in the heart, by overexpressing the phosphatase dPTEN or the forkhead transcription factor dFOXO, prevents the decline in cardiac performance with age. Thus, insulin-IGF signaling influences age-dependent organ physiology and senescence directly and autonomously, in addition to its systemic effect on lifespan. The aging fly heart is a model for studying the genetics of age-sensitive organ-specific pathology.

Nature Genetics, 36, 1275-1281.

Long-lived flies maintain the functional heart.

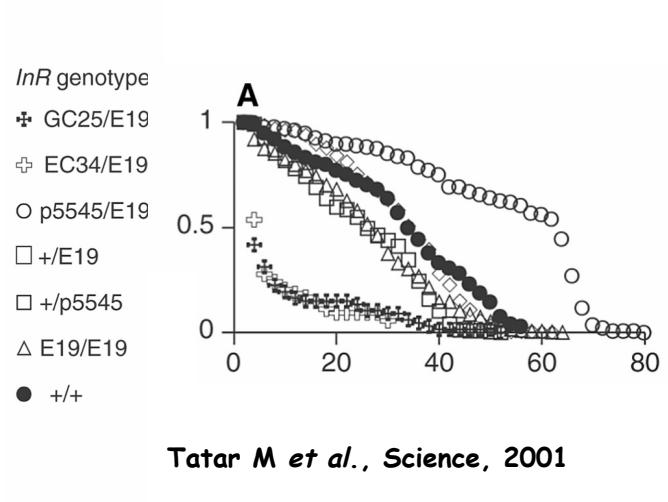
-The evolutionary conservation of longevity-determination pathway
-Mutations in insulin receptor (*InR*) and insulin receptor substrate (*chico*) extend life span.

The Insulin/IGF-I signaling pathway



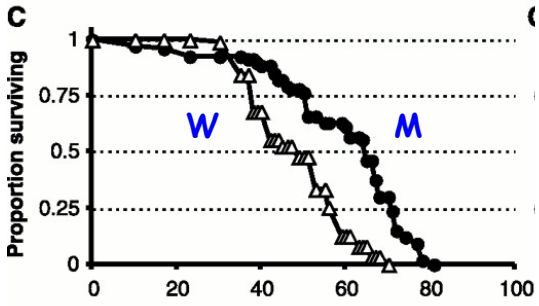
Barbieri M *et al.*, Am J Physiol Endocrinol Metab, 2003

***InR* mutant**



Tatar M *et al.*, Science, 2001

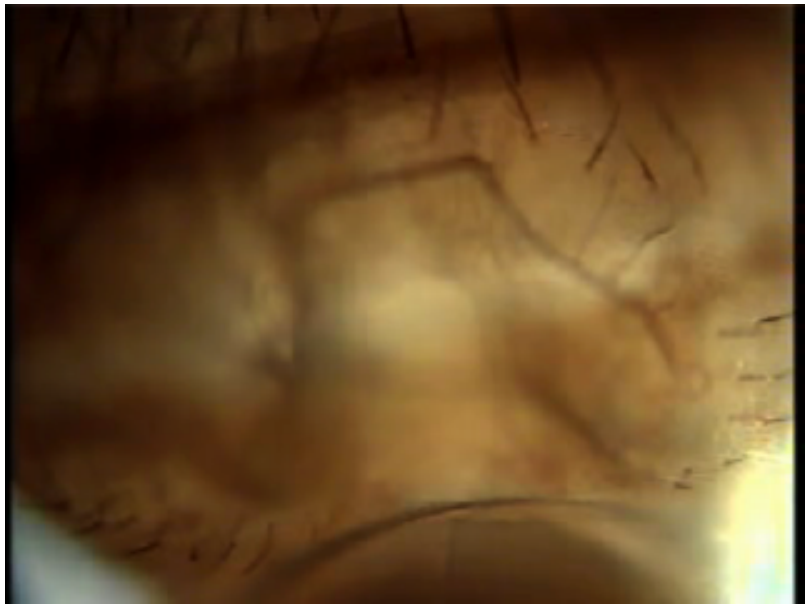
***chico* mutant**



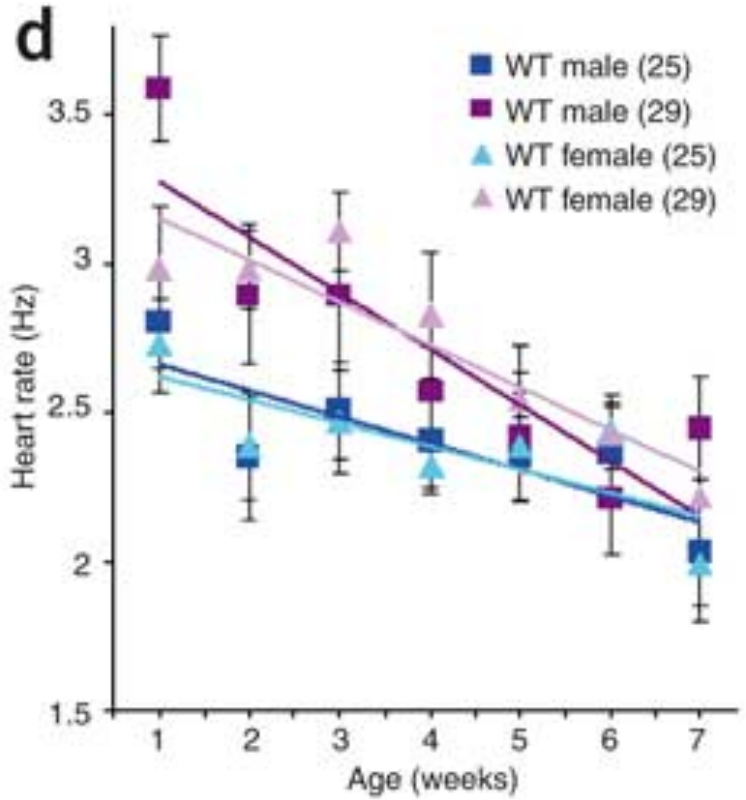
Clancy D *et al.*, Science, 2001

Measuring heart rate in *Drosophila*

After flies are anaesthetized with FlyNap (triethylamine), the heart rate is measured between diastole and systole by visually counting for 20 seconds.



D. melanogaster heart rate changes with age.

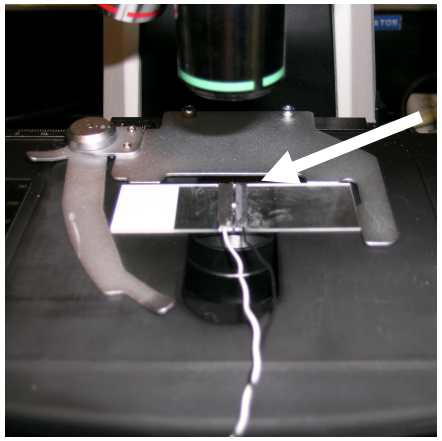


D. melanogaster heart rate is declined with aging.

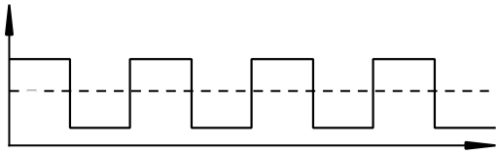
Measuring heart failure rate in *Drosophila*

Electrical pacing: After we pace the heart at 4 V and 6 Hz for 30 seconds, the heart failure rate is defined as the percentage of flies that enter a cardiac arrest.

Pacing Setup

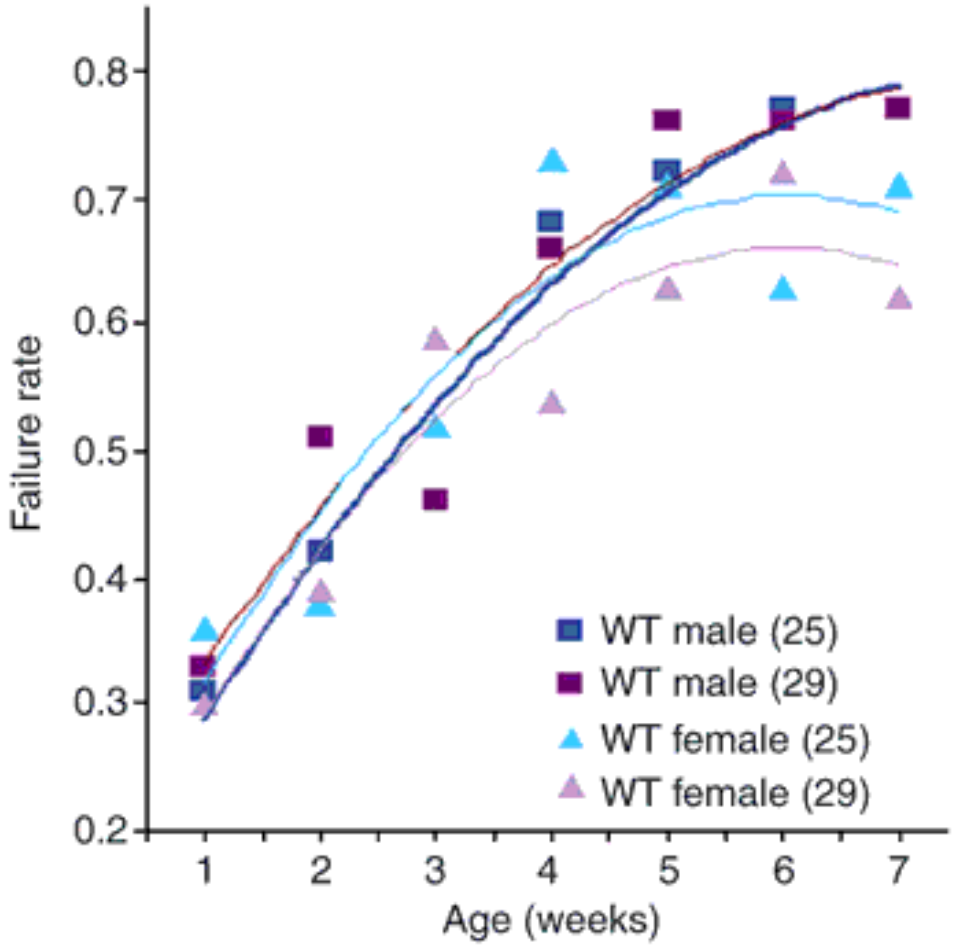


Pacing Slide Constructed With Lead Sheets



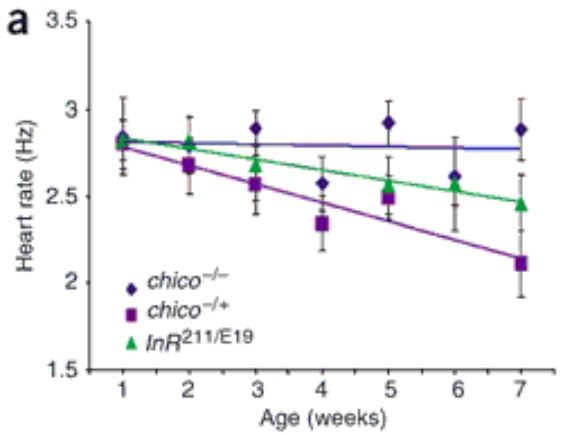
5-9 beats/sec Pacing Induced with 4V Square Wave

Heart failure as a function of age after external electrical pacing.



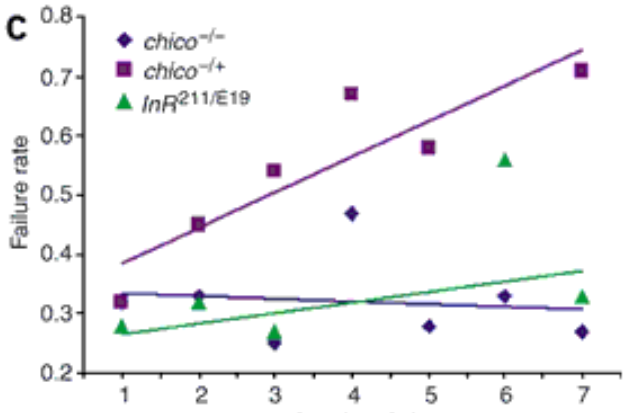
Heart failure rate is increased with aging.

Long-lived flies (*InR-* and *chico-*) show consistent heart rate and low heart failure with aging.



Long-lived flies

Wild type



Wild type

Long-lived flies

Wessells R *et al.*, Nat Genet, 2004

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(http://njms.umdnj.edu/departments/cell_biology_and_molecular_medicine/index.cfm)