有翅膀的小人類-

果蠅的醫學研究

中正大學 生科系
黃敏郎

Model organism (模式生物)

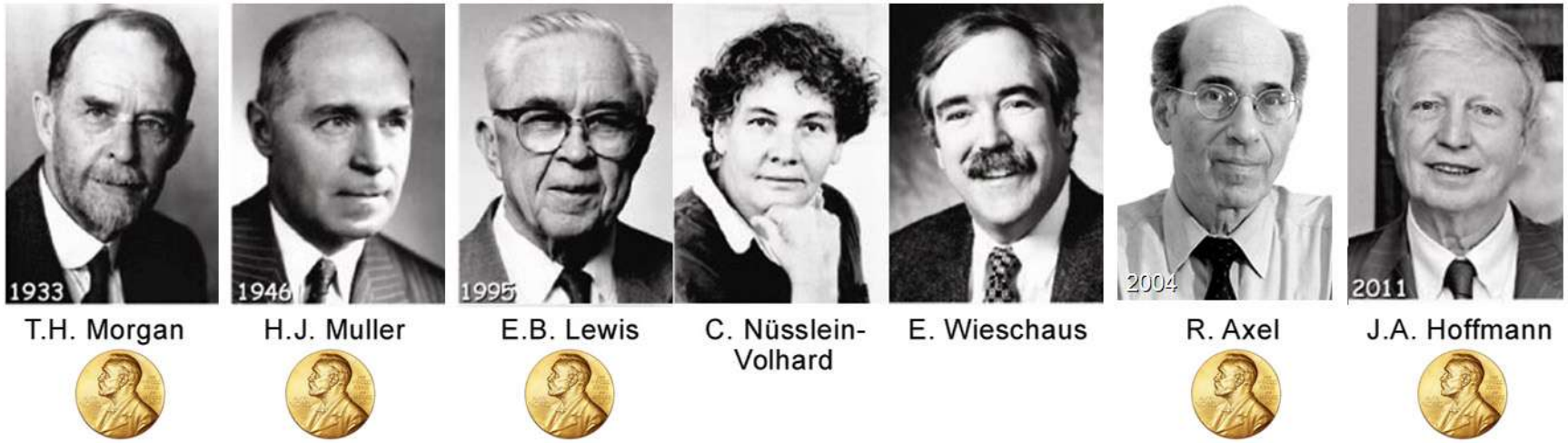
- Grow quickly
- Relatively simple
- Inexpensive
-

Drosophila melanogaster (果蠅)

http://www.genomenewsnetwork.org/articles/05_02/sleepless_flies.shtml

- Short life cycle
- Ease of culture and maintenance
- Small genome size
- Extremely low cost of maintenance, propagation, and screening and the rapidity of studies in the fly compared with traditional mammal-based models.

Nobel prizes in physiology or medicine



Chromosome

X-ray mutagenesis

Embryo development and Hox genes

Olfactory system

Innate immunity

https://droso4schools.files.wordpress.com/2015/04/nobellaur-large2.jpg

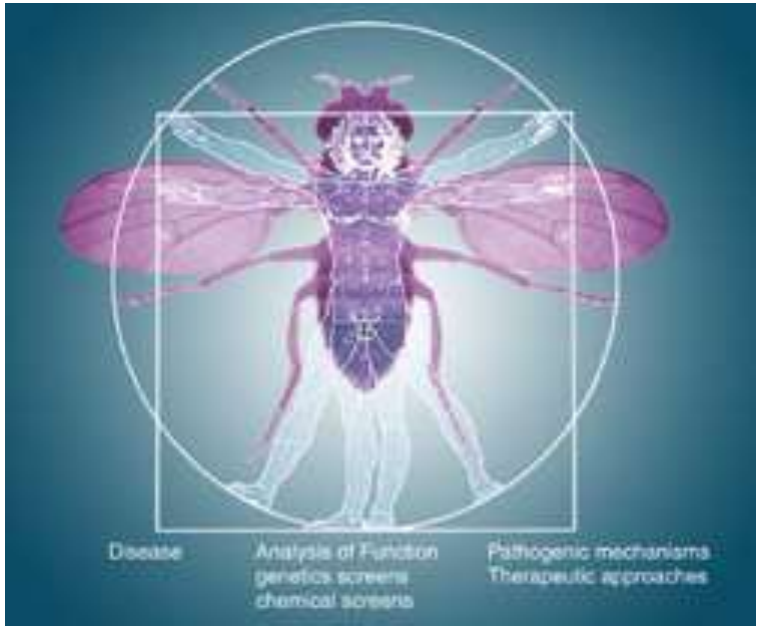
Drosophila research on human disease

Human

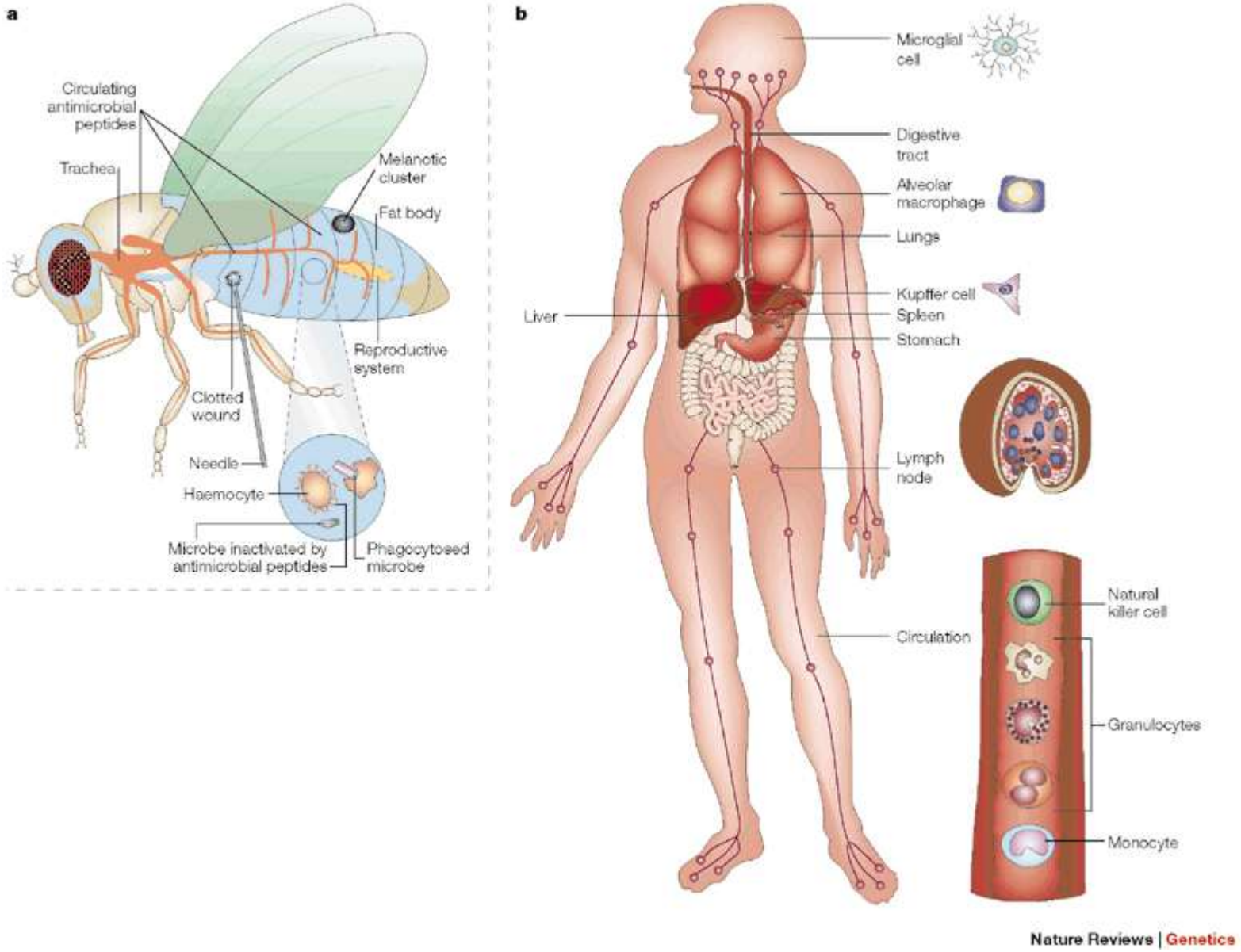
Mechanism?

Drug?

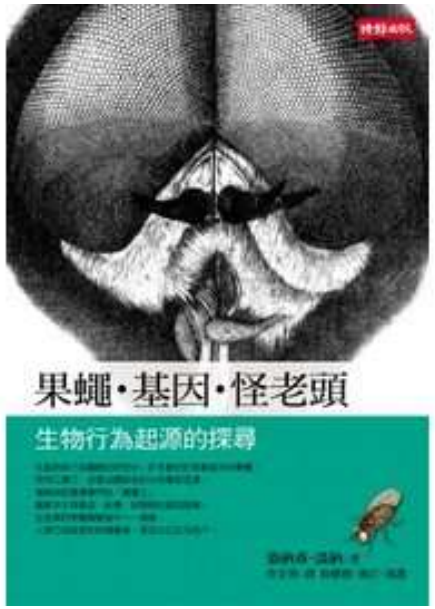
Disease

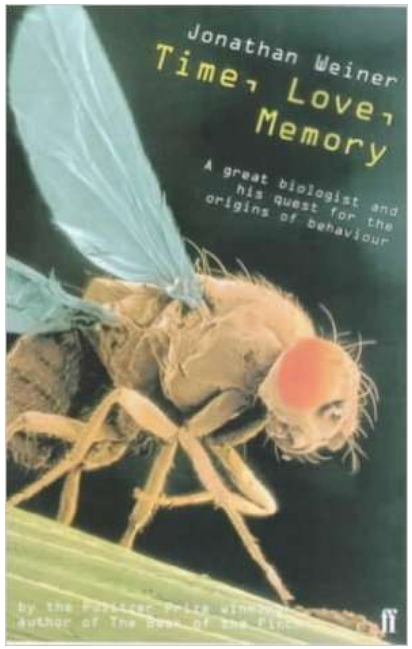


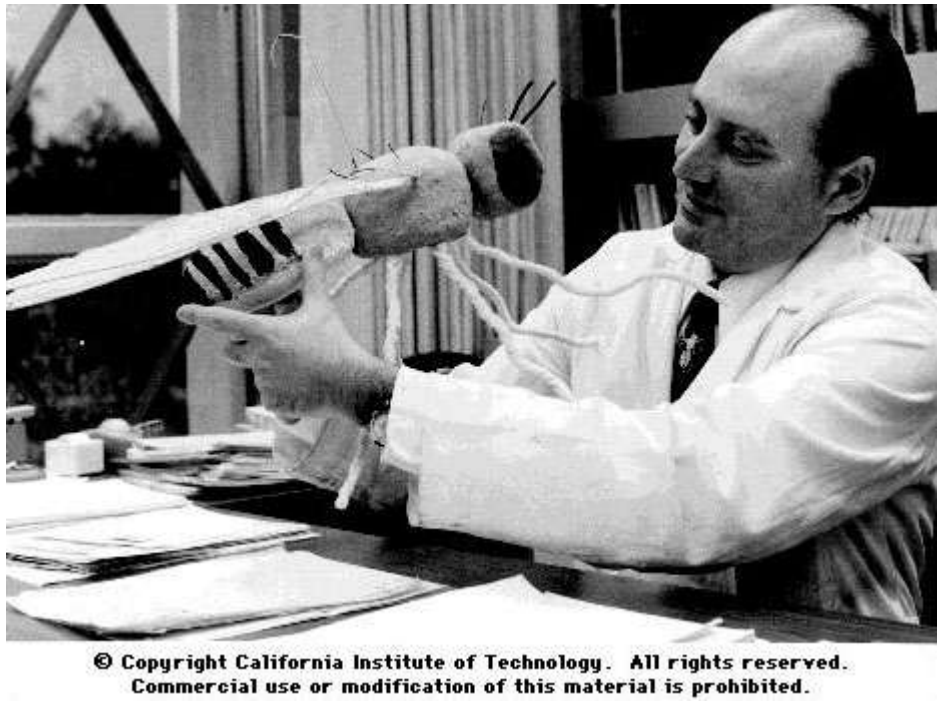
http://www.nature.com/ng/journal/v39/n5/images/ng0507-589-I1.jpg



http://www.nature.com/nrg/journal/v2/n4/fig_tab/nrg0401_256a_F1.html





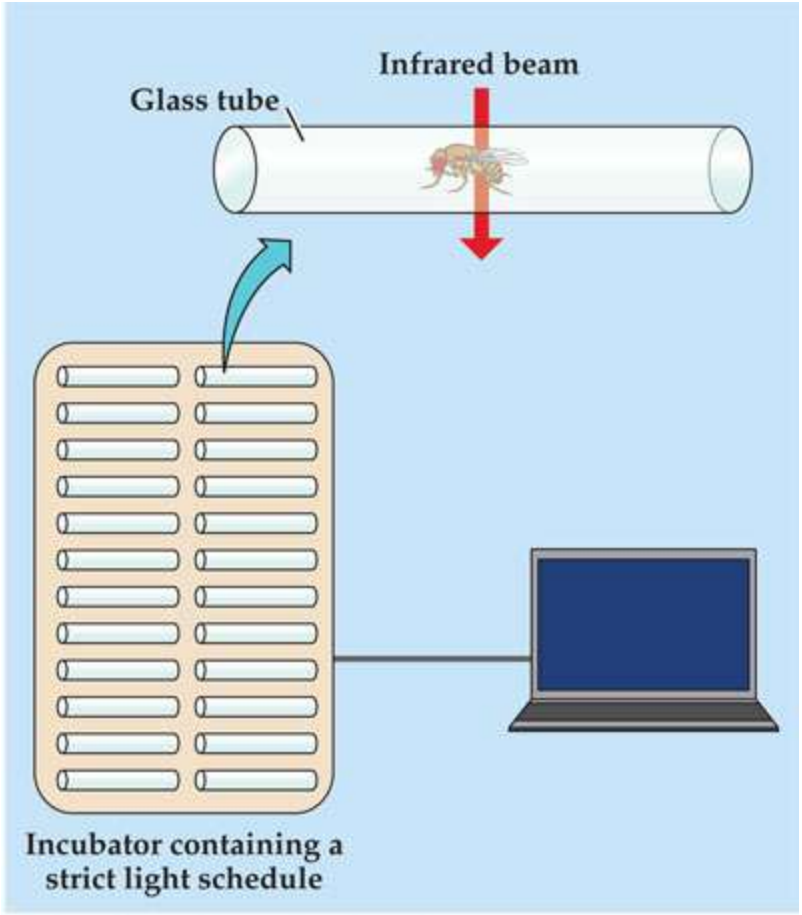


Seymour Benzer

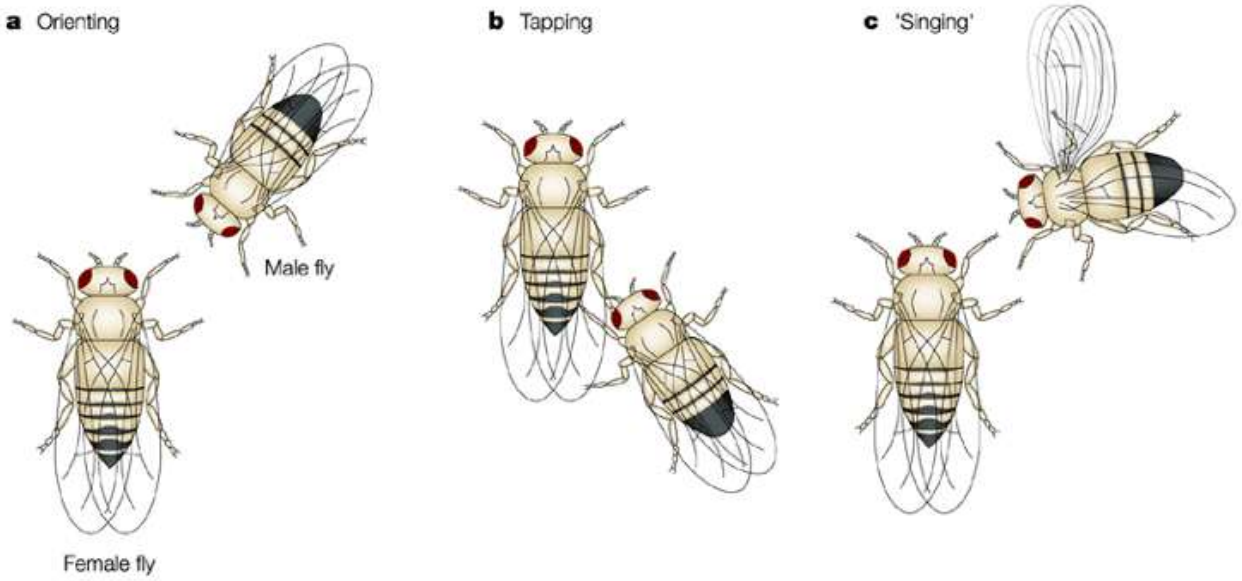
http://img.pcstore.com.tw/~prod/M15725320_big.jpg?pimg=static&P=1428467225

http://50annidna.scienze.unipd.it/DFTB/concept_27_ITA/images/benzerfly.jpg

https://images-na.ssl-images-amazon.com/images/I/41XSEFNYF7L.jpg



http://sites.sinauer.com/animalphys3e/boxex/AnPhys3e-BoxEx-15-03-C-0.jpg

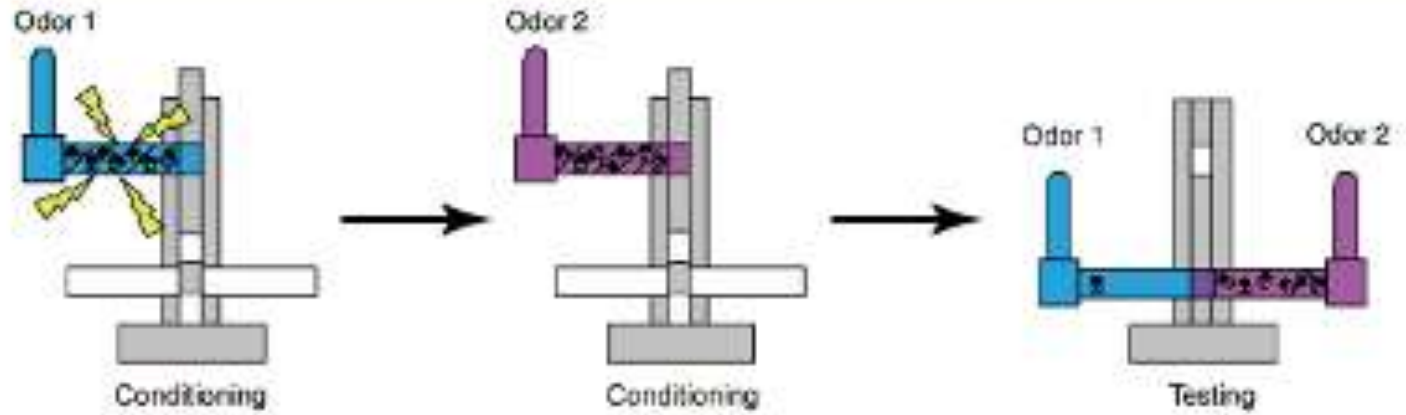


Mating (micro CT)

http://3.bp.blogspot.com/-H3bzQrNCyY0/UuZl8tagZvI/AAAAAAAACc8/AFuoEUmC-GU/s1600/5-courtship.gif

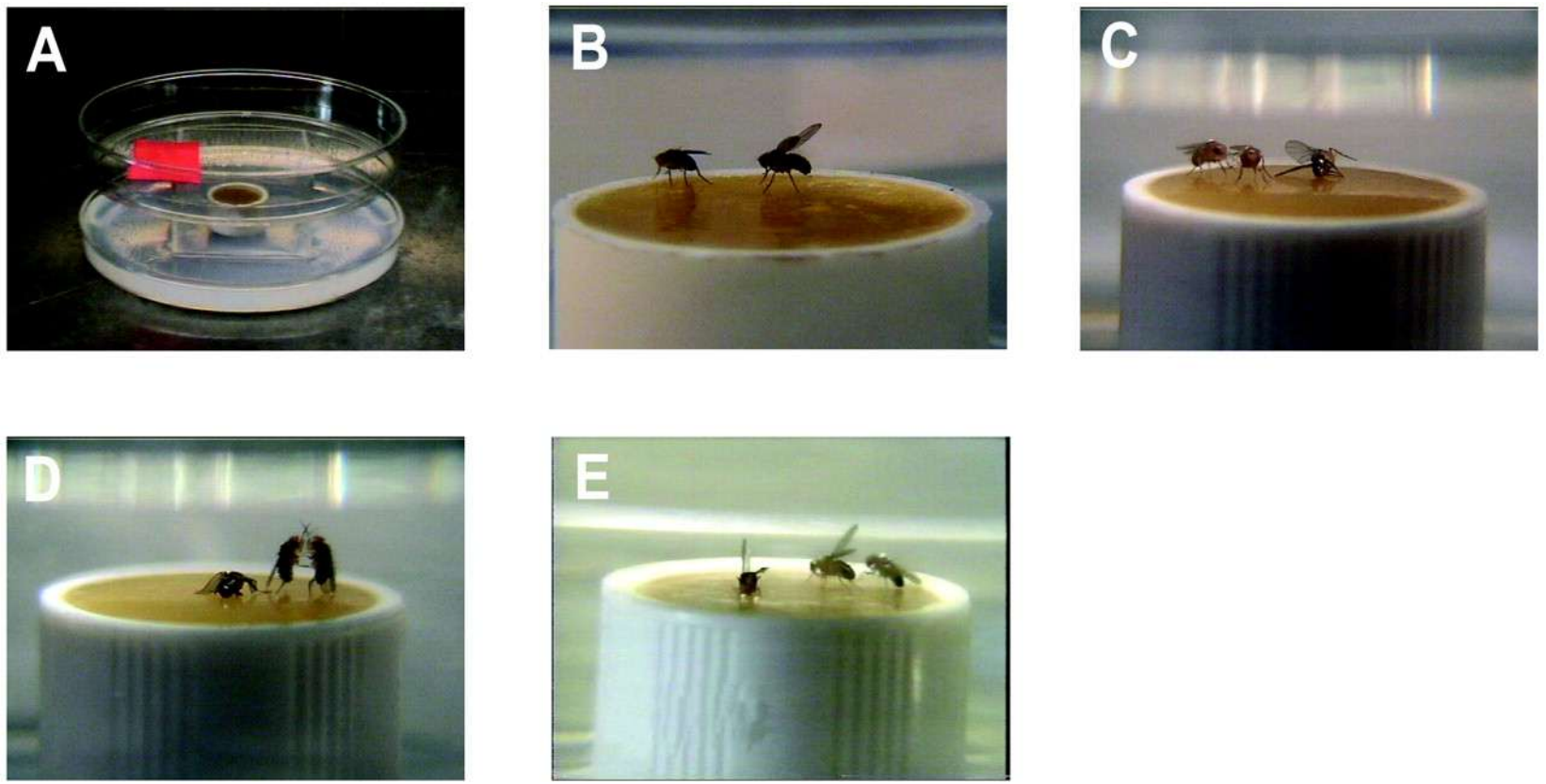
http://news.cornell.edu/stories/2015/06/3-d-scans-mating-fruit-flies-uncover-female-biology

Olfactory aversive conditioning and testing

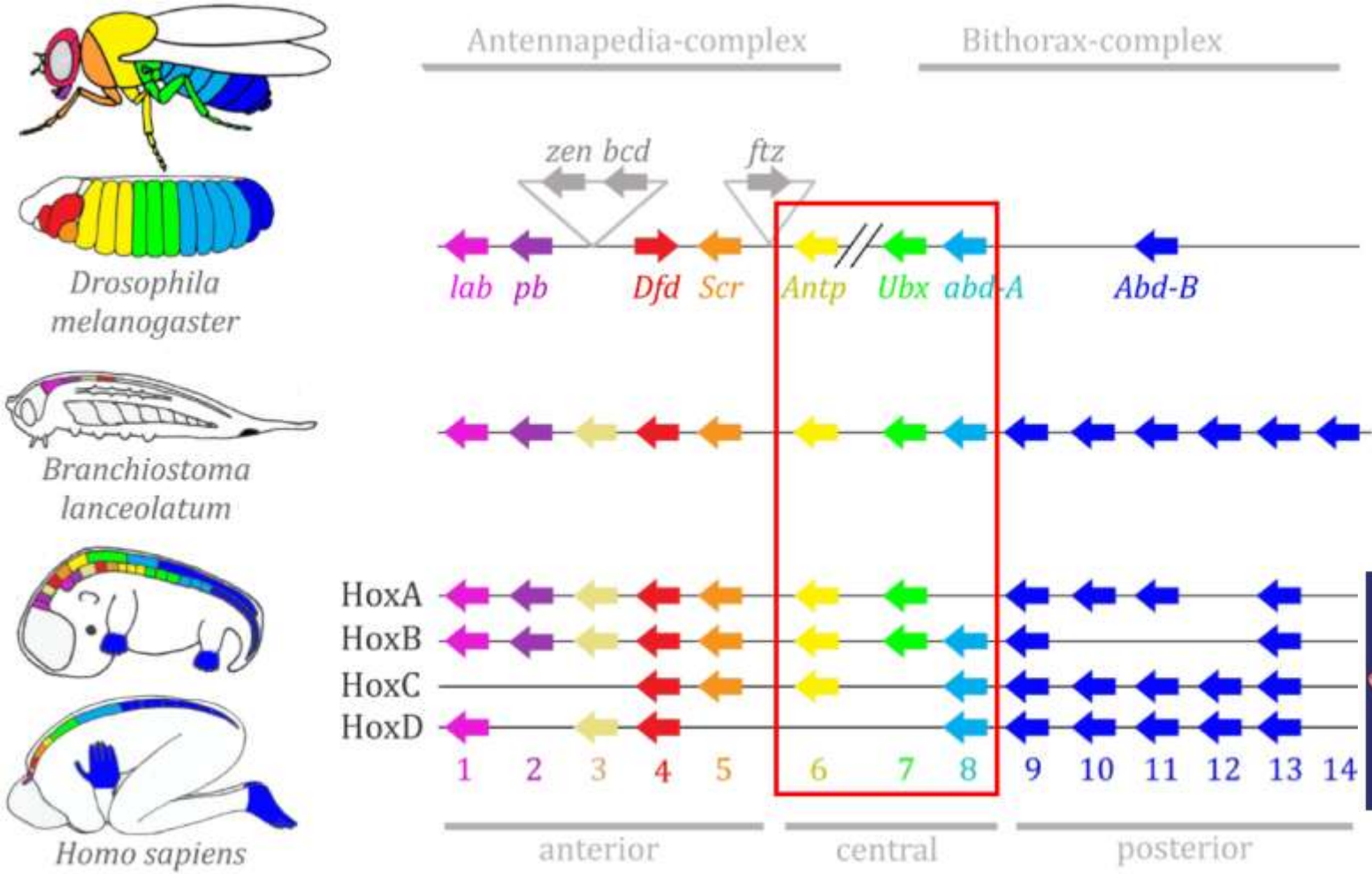


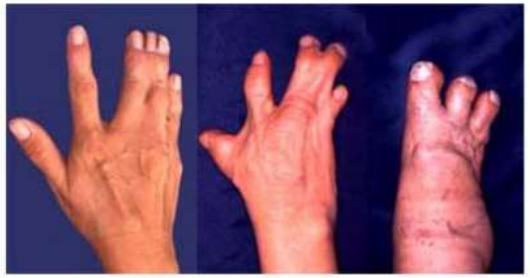
http://www.united-academics.org/magazine/wp-content/uploads/2015/02/procedure.jpg

Experimental chamber and components of fruit fly fighting.



Selby Chen et al. PNAS 2002;99:5664-5668





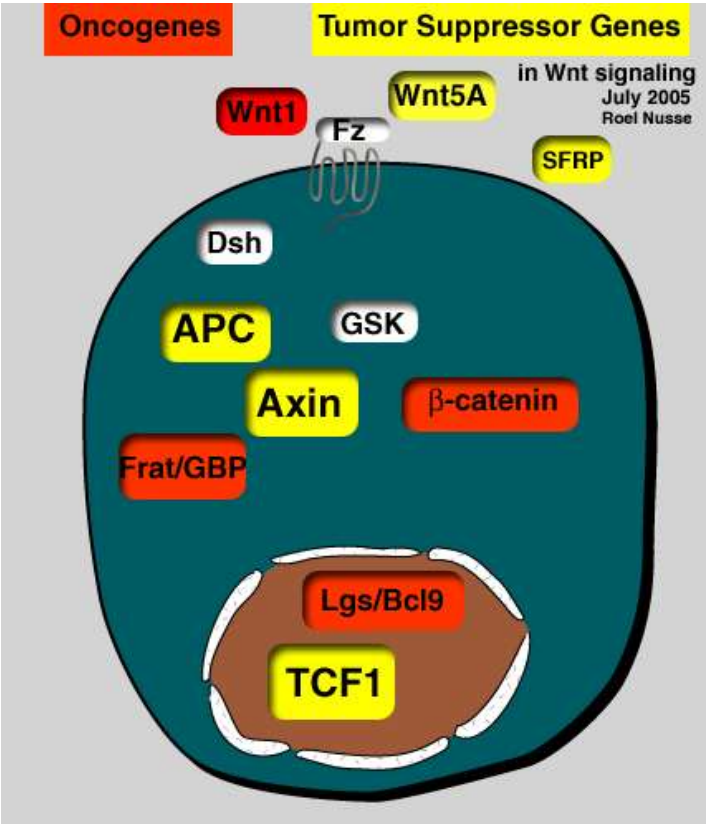
Synpolydactyly phenotype with mutation in HOXD13 (+10 Ala expansion) in heterozygous (left) and homozygous (hand and foot) individuals.

http://bioinformatics.uni-konstanz.de/HueberHox/Pics/Hox_Evolution_central_old.png

http://courses.biology.utah.edu/bastiani/3230/db%20lecture/lectures/Limb/HumanHoxHand.jpg

Conserved signal pathways

- EGFR (Ras)
- Wingless/Wnt
- Notch



http://stanford.edu/~rnusse/figs/oncog.gif

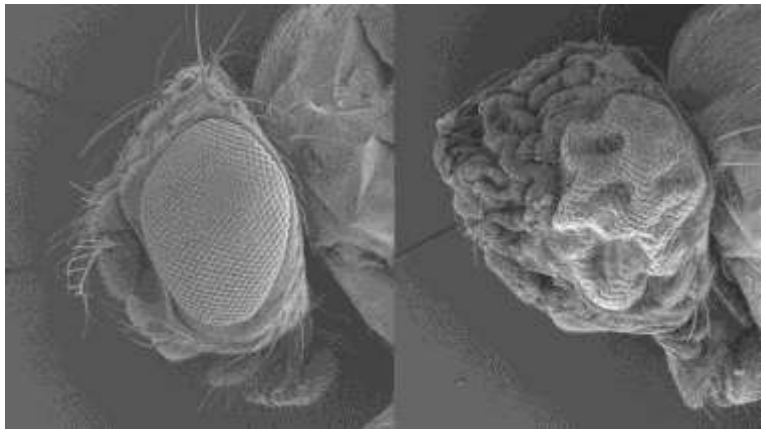
Table 1. Pathways associated with human congenital disorders

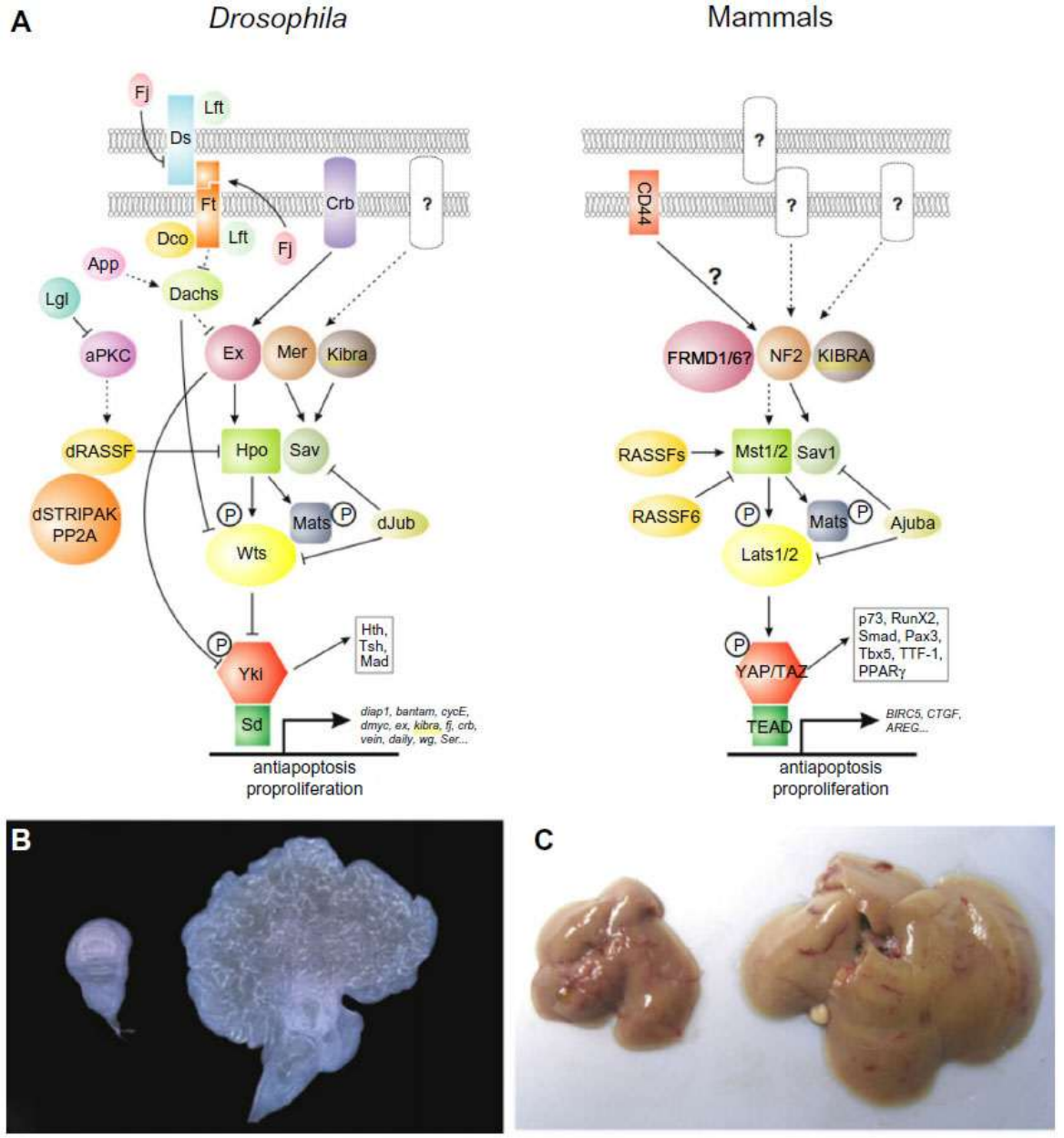
Pathway[1]	Disease	Phenotype MIM no.[2]	Human causal gene	*Drosophila* ortholog[3]
Notch	Alagille syndrome	610205	*NOTCH2*	*N*
			JAG1	*Ser*
	Congenital heart disease	600001	*JAG1*	*Ser*
	Tetralogy of Fallot	187500	*JAG1*	*Ser*
	Adams-Oliver syndrome 5	616028	*NOTCH1*	*N*
	Hajdu-Cheney syndrome	102500	*NOTCH2*	*N*
	Myofibromatosis, infantile 2	615293	*NOTCH3*	*N*
	Lateral meningocele syndrome	130720	*NOTCH3*	*N*
	Spondylocostal dysostosis 1	277300	*DLL3*	*dl*
Wnt/PCP	Van Maldergem syndrome	615546	*DCHS1*	*ds*
			FAT4	*ft*
	Exudative vitreoretinopathy 1	133780	*LRP5*	*arr*
			FZD4	*fzd*
			NDP	Unknown
	Hennekam lymphangiectasia-lymphedema syndrome 2	616006	*FAT4*	*ft*
	Robinow syndrome, autosomal dominant 2	616331	*DVL1*	*dsh*
	Mental retardation, autosomal dominant 19	615075	*CTNNB1*	*arm*
	Tetra-amelia syndrome	273395	*WNT3*	*wg*
	Mullerian aplasia and hyperandrogenism	158330	*WNT4*	*wg*
	SERKAL syndrome	611812	*WNT4*	*wg*
	Fuhrmann syndrome	228930	*WNT7A*	*wg*
	Odontoonychodermal dysplasia	257980	*WNT10A*	*wg*
	Split-hand/foot malformation 6	225300	*WNT10B*	*wg*
	Caudal duplication anomaly	607864	*AXIN1*	*axn*
	Tooth agenesis, selective, 4	150400	*AXIN2*	*axn*

- Hippo pathway

→ Size control

→ Tumor suppression





http://scienceblog.cancerresearchuk.org/2014/03/13/science-snaps-why-arent-flies-as-big-as-hippos/ https://www.dovepress.com/cr_data/article_fulltext/s48000/48505/img/fig1.jpg

Advantage

- 13000 genes, including the counterparts of 65% human disease-causing genes
- Shares many similar features and pathways with humans
- Lack of genetic redundancy
- Bypass some of the ethical issues of biomedical research involving human subjects
- Powerful genetic tools

Powerful genetic tools

- Mutants
- GAL4/UAS
- Mosaic analysis
- Time and tissue specific inducible promoters are available
- UAS-RNAi
- EP lines

樂於合作與分享的果蠅社群

- FlyBase
- Stock centers
- FlyCore (臺灣果蠅遺傳資源中心)

台灣的果蠅實驗室 > 50

- 中研院 13
- 台大 10
- 師大 1
- 國防醫 2
- 陽明 2
- 長庚 4
- 中央 1
- 清大 5
- 交大 1
- 國衛院 2
- 東海 2
- 中國醫 2
- 暨南 1
- 中正 1
- 成大 4
- 慈濟 1

http://flycoretw.lifescience.ntu.edu.tw/%E5%85%B6%E4%BB%96%E8%B3%87%E8%A8%8A.html

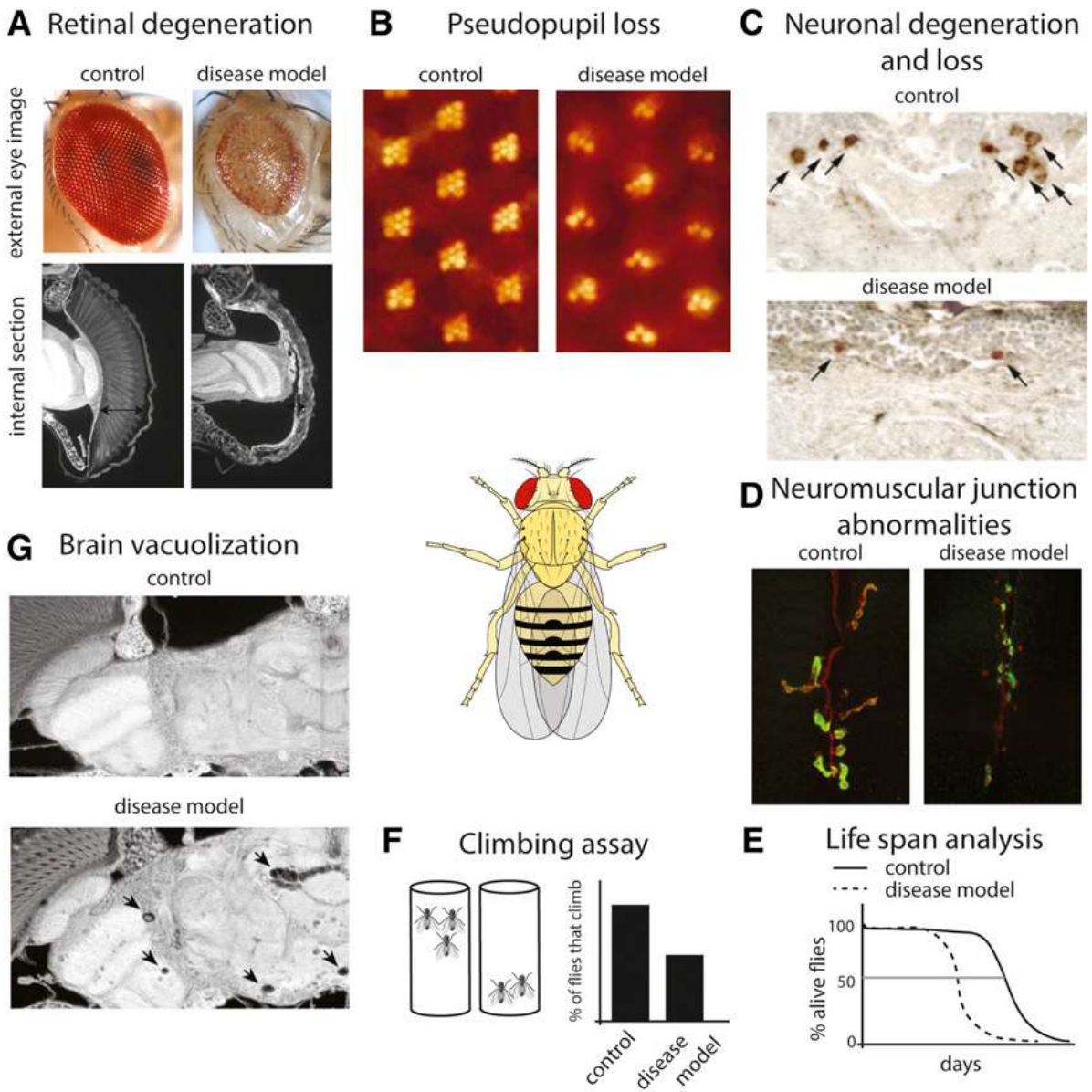
Limitation

- No model organisms is expected to faithfully replicate the human disease phenotype

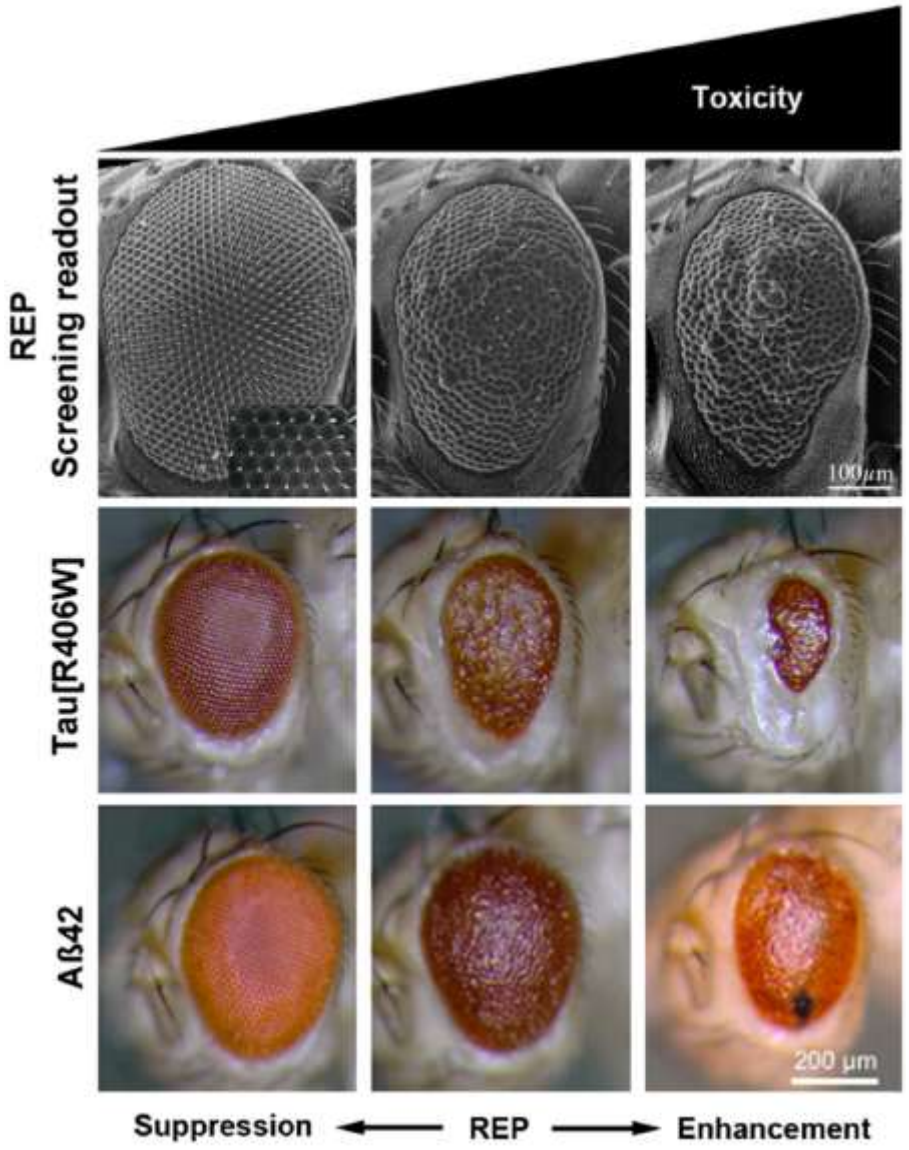
- Vertebrate- or human-specific factors, cells, tissues, or systems

- Overexpression of disease gene

→ Disease model



- Modifier screen



- Alzheimer’s disease (Aβ)
- Polyglutamine expansion disorders

  Huntington’s disease (Htt)

  Spinocerebellar ataxias 3 (小脳萎縮症)(Ataxin-3)

  Amyotrophic lateral sclerosis (肌萎縮性側索硬化; 漸凍人)

→ Modifiers include chaperone and ubiquitin-proteasome pathway

Nat Genet. 1999 Dec;23(4):425-8.

Suppression of polyglutamine-mediated neurodegeneration in Drosophila by the molecular chaperone HSP70.

Warrick JM[1], Chan HY, Gray-Board GL, Chai Y, Paulson HL, Bonini NM.

Author information

[1]Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania, USA.

Abstract

At least eight inherited human neurodegenerative diseases are caused by expansion of a polyglutamine domain within the respective proteins. This confers dominant toxicity on the proteins, leading to dysfunction and loss of neurons. Expanded polyglutamine proteins form aggregates, including nuclear inclusions (NI), within neurons, possibly due to misfolding of the proteins. NI are ubiquitinated and sequester molecular chaperone proteins and proteasome components, suggesting that disease pathogenesis includes activation of cellular stress pathways to help refold, disaggregate or degrade the mutant disease proteins. Overexpression of specific chaperone proteins reduces polyglutamine aggregation in transfected cells, but whether this alters toxicity is unknown. Using a Drosophila melanogaster model of polyglutamine disease, we show that directed expression of the molecular chaperone HSP70 suppresses polyglutamine-induced neurodegeneration in vivo. Suppression by HSP70 occurred without a visible effect on NI formation, indicating that polyglutamine toxicity can be dissociated from formation of large aggregates. Our studies indicate that HSP70 or related molecular chaperones may provide a means of treating these and other neurodegenerative diseases associated with abnormal protein conformation and toxicity.

SCIENTIFIC REPORTS

OPEN

Drosophila screen connects nuclear transport genes to DPR pathology in c9ALS/FTD

Received: 07 August 2015
Accepted: 11 January 2016
Published: 12 February 2016

Steven Boeynaems[1,2,*], Elke Bogaert[1,2,*], Emiel Michiels[1,2], Ilse Gijselinck[3,4], Anne Sieben[3,4,5], Ana Jovičić[6], Greet De Baets[7,8], Wendy Scheveneels[1,2], Jolien Steyaert[1,2], Ivy Cuijt[3,4], Kevin J. Verstrepen[9,10], Patrick Callaerts[11,12], Frederic Rousseau[7,8], Joost Schymkowitz[7,8], Marc Cruts[3,4], Christine Van Broeckhoven[3,4], Philip Van Damme[1,2,13], Aaron D. Gitler[6], Wim Robberecht[1,2,13] & Ludo Van Den Bosch[1,2]

Hexanucleotide repeat expansions in *C9orf72* are the most common cause of amyotrophic lateral sclerosis (ALS) and frontotemporal degeneration (FTD) (c9ALS/FTD). Unconventional translation of these repeats produces dipeptide repeat proteins (DPRs) that may cause neurodegeneration. We performed a modifier screen in *Drosophila* and discovered a critical role for importins and exportins, Ran-GTP cycle regulators, nuclear pore components, and arginine methylases in mediating DPR toxicity. These findings provide evidence for an important role for nucleocytoplasmic transport in the pathogenic mechanism of c9ALS/FTD.

Amyotrophic lateral sclerosis (ALS) and frontotemporal degeneration (FTD)

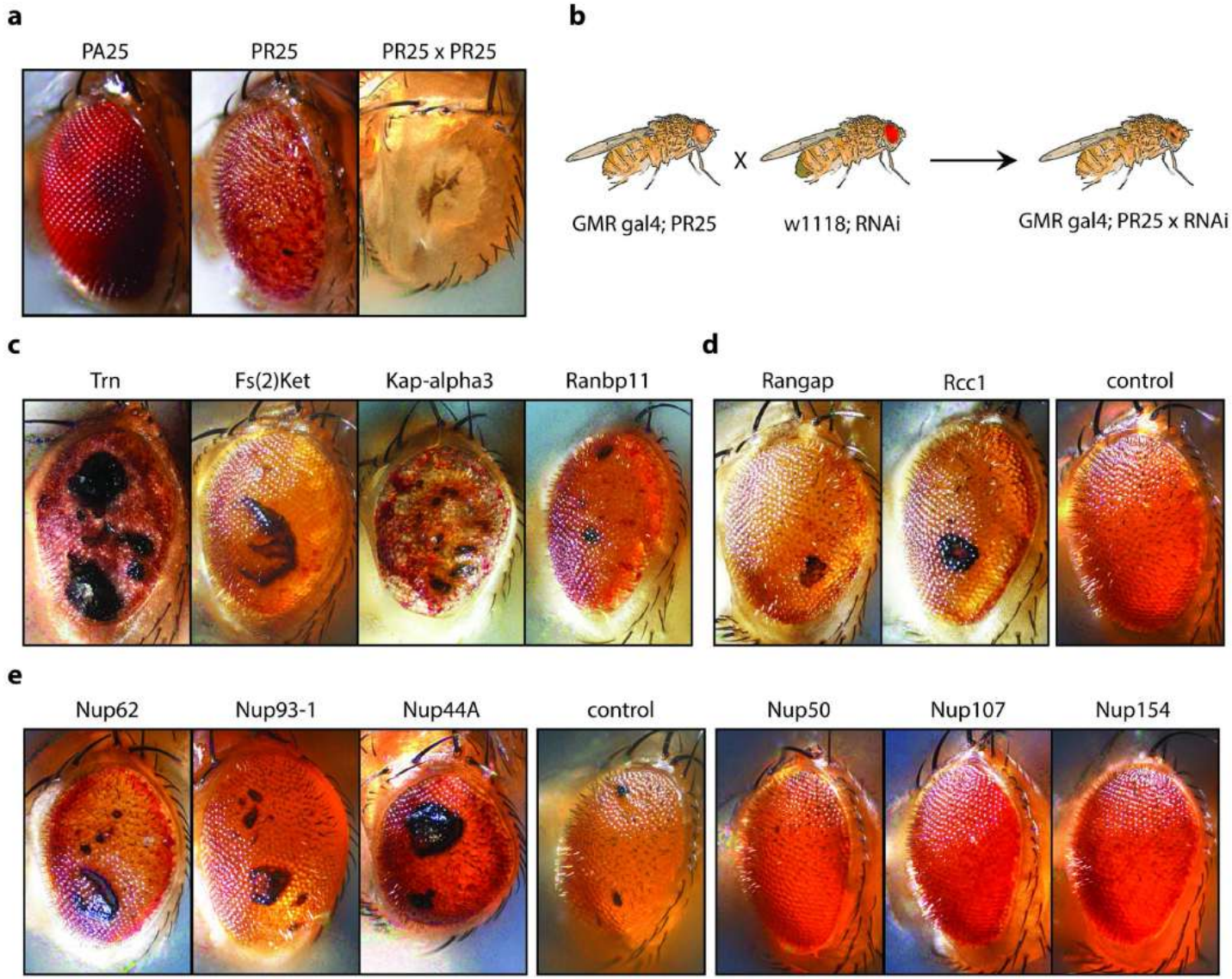
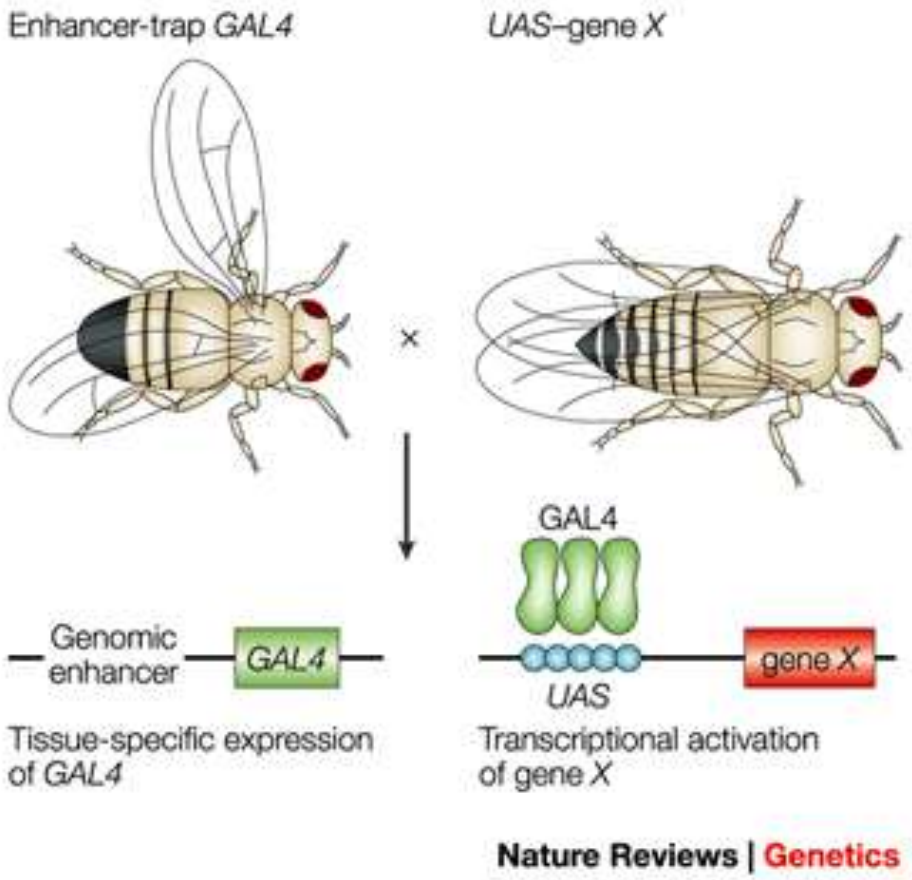


Figure 1. Genes implicated in nuclear transport are potent modifiers of PR toxicity in *Drosophila*.



http://www.nature.com/nrg/journal/v3/n3/box/nrg751_BX2.html

- Drugs can modify the phenotypes.

- 薑黃素

SCIENTIFIC REPORTS

OPEN

Received: 31 July 2015
Accepted: 25 November 2015
Published: 05 January 2016

Curcumin modulates cell death and is protective in Huntington's disease model

Anjalika Chongtham & Namita Agrawal

Huntington's disease (HD) is a progressive, dominantly inherited neurological disorder caused by an abnormal expansion of polyglutamine (polyQ) repeat within the Huntingtin (Htt) protein with no disease modifying treatments. In a *Drosophila* model of HD, expression of mutant Huntingtin (Htt) protein with expanded polyQ leads to formation of inclusion bodies (IBs), increase in cellular toxicity, progression of motor disabilities and reduced viability. Multiple cellular events such as oxidative stress, mitochondrial dysfunction, inflammation and transcriptional dysregulation are reported to contribute to pathology, however, till date there are no disease-modifying treatments with least side effects. Therefore, we investigated effect of the phytochemical curcumin on HD pathogenesis. Curcumin, a phytochemical and commonly used ingredient in Asian food has a wide spectrum of anti-oxidant, anti-inflammatory and anti-fibrilogenic properties. In this study, we provide evidence that curcumin significantly ameliorates disease symptoms in a *Drosophila* model of HD by suppressing cell death and can be a key to halting the progression of Huntington's disease with least side effects.

Problem of drug screens

- For example, a recent screen of 184,880 novel compounds using a “filter retardation assay” of Huntington's disease (HD) aggregates led to the identification of multiple lead compounds, including a number of benzothiazoles that inhibited polyglutamine-mediated aggregation of toxic and misfolded proteins.
- Because riluzole, a closely related benzothiazole, had previously shown therapeutic benefit in patients with amyotrophic lateral sclerosis drugs from this structural class of molecules were tested for further development.
- In a cell culture model of aggregation, all primary hits were found to be toxic to cells, and in an animal model of HD, *none* of the compounds was of therapeutic.

- High-throughput screening
- Fully automated scoring of a visible phenotype, either live/dead, or a visible marker.

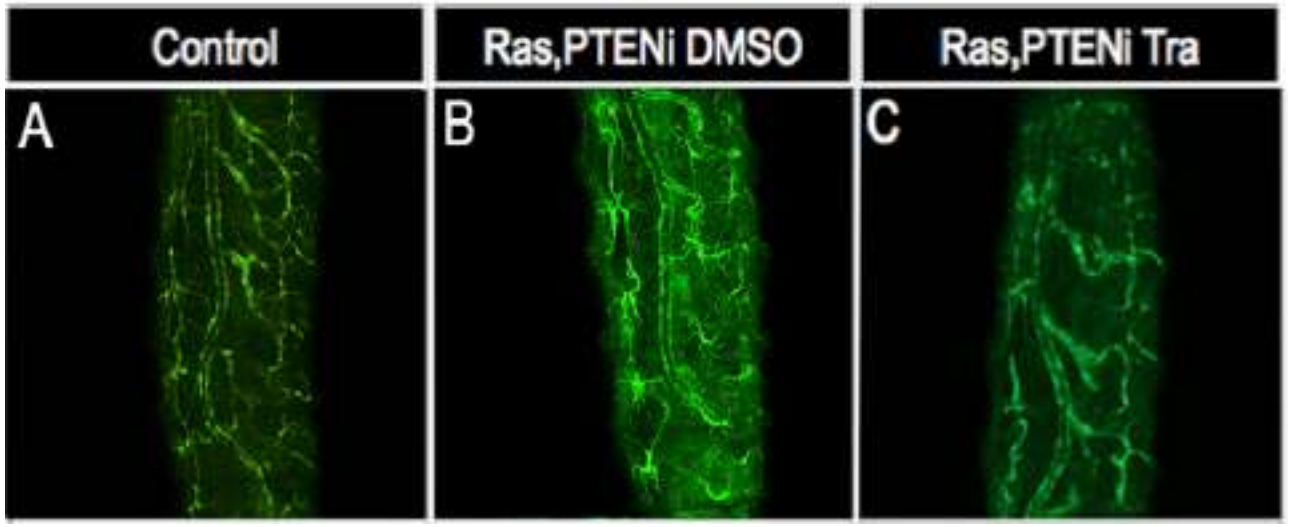
→ fly!

Cell Reports
Article

Drosophila Lung Cancer Models Identify Trametinib plus Statin as Candidate Therapeutic

Benjamin D. Levine[1] and Ross L. Cagan[1,*]
[1]Department of Developmental and Regenerative Biology and the Graduate School of Biomedical Sciences, Icahn School of Medicine at Mount Sinai, One Gustave Levy Place, New York, NY 10029-1020, USA

Screen
1,192 FDA-approved drugs



Trametinib: cancer drug; MEK inhibitor

Statin: lipid-lowering; HMG-CoA reductase inhibitors

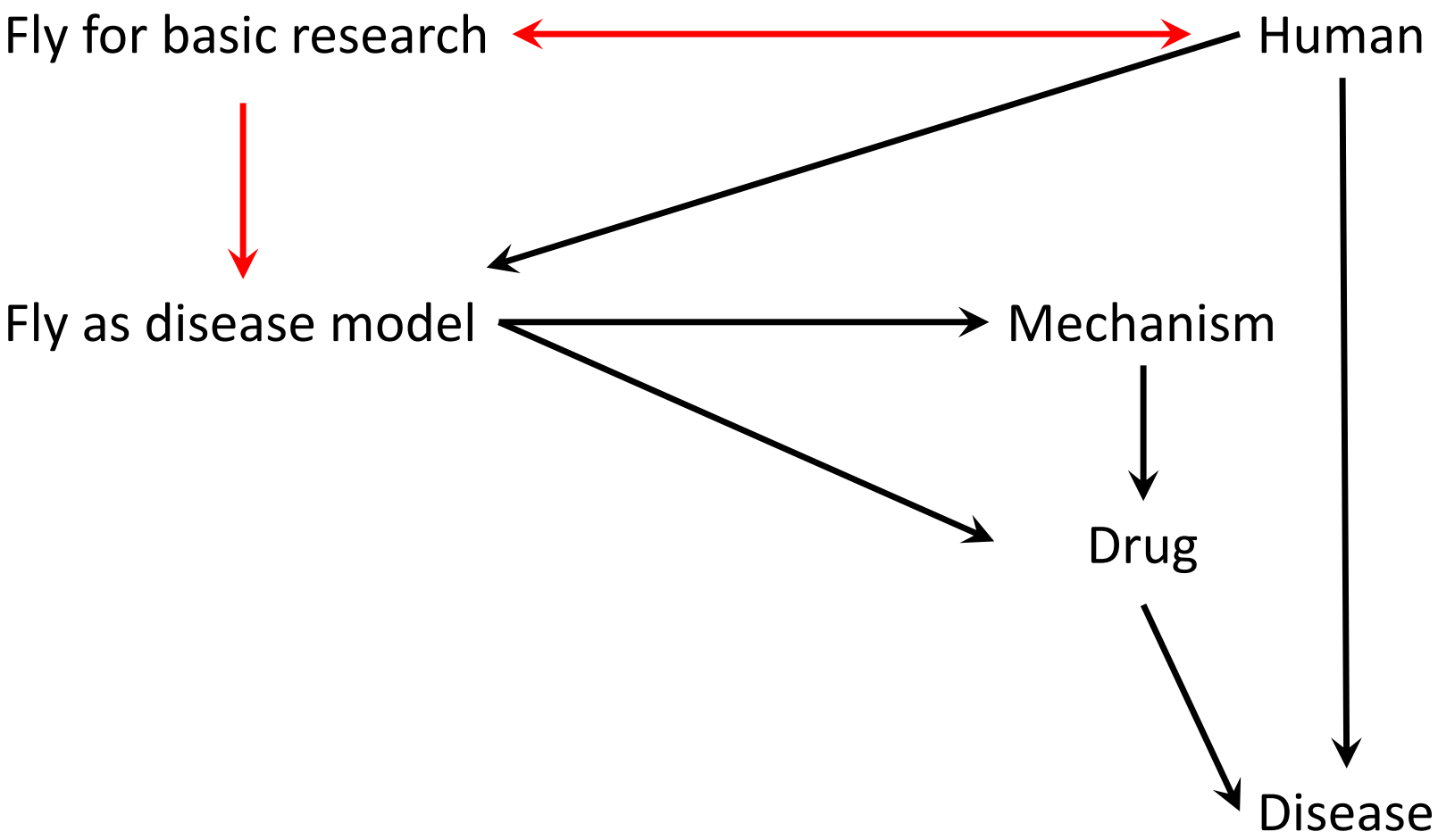
From a human disease gene to the fly homolog

- Studying the fly's homolog of the disease gene

  parkin for Parkinson's disease

From a fly gene to human disease

- study of a fly gene
- → mutant phenotype
  → whether its human homolog is a disease gene?
  → the mutant can be the disease model


Fly for basic research
Human
Fly as disease model
Mechanism
Drug
Disease

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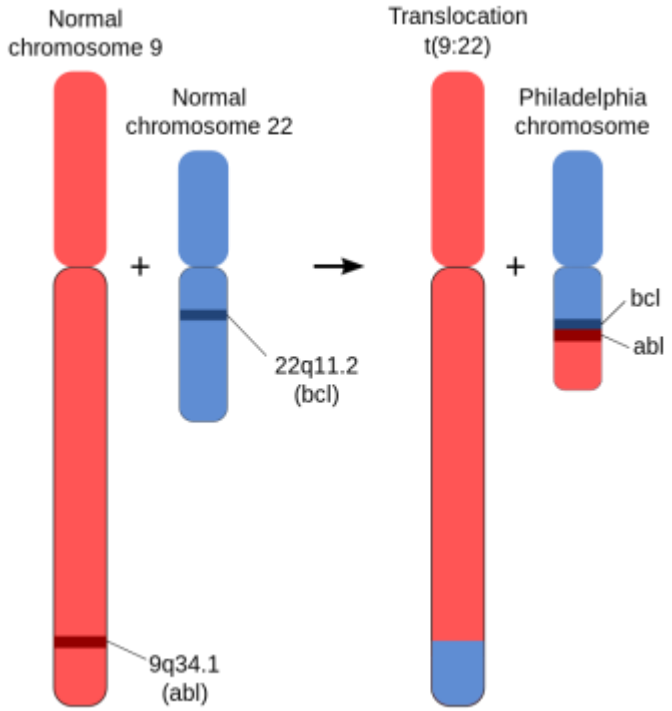
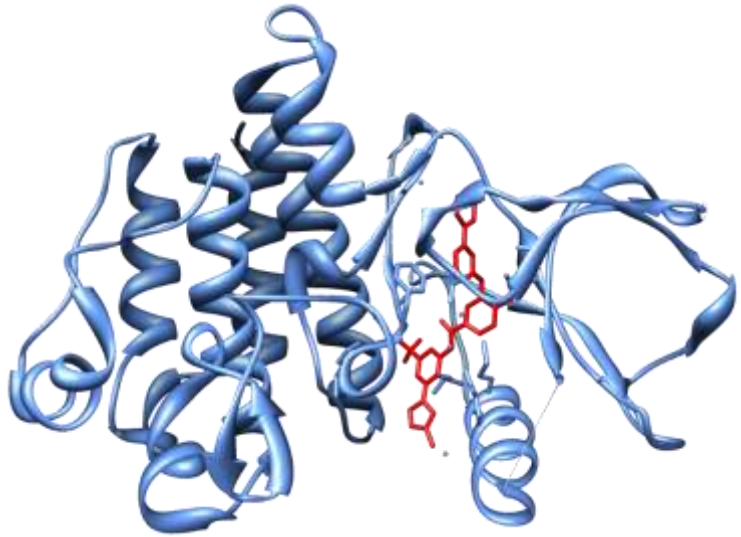


Philadelphia chromosome



Crystal structure of Abl kinase domain (blue) in complex with 2nd generation tyrosine kinase inhibitor (TKI) nilotinib(red)

https://en.wikipedia.org/wiki/Philadelphia_chromosome