Qué ofrece Drosophila como modelo de estudio de las enfermedades neurodegenerativas

3º Curso de Genética Humana. SEG

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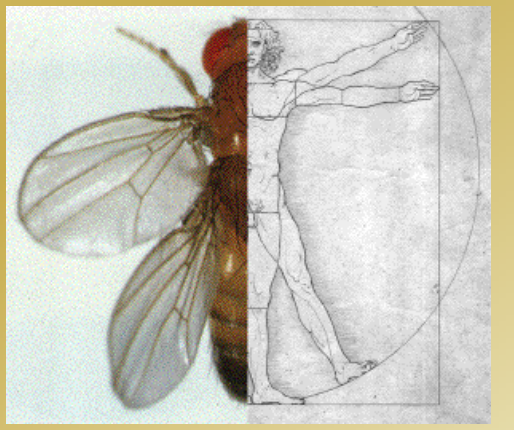
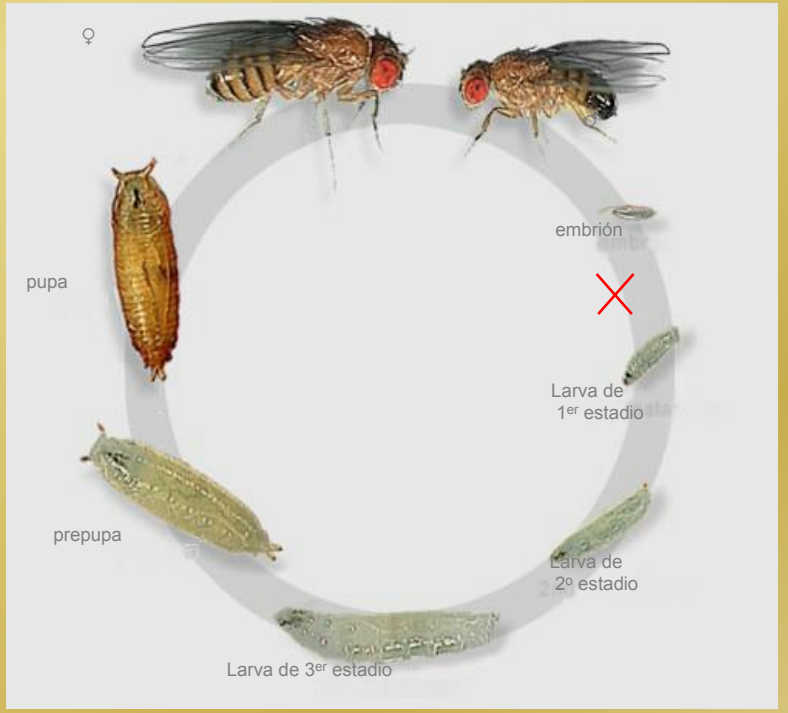


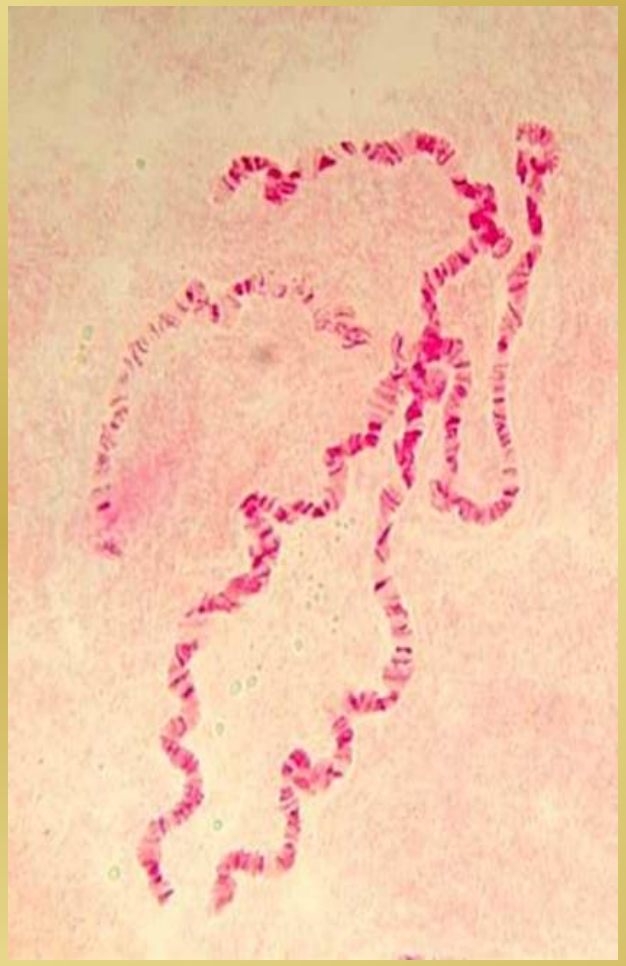
- Facilidad de cultivo y mantenimiento en el laboratorio
- Bajo coste
- Tiempo de generación corto y abundante descendencia

Drosophila melanogaster

- Desarrollo postembrionario indirecto
- Metamorfosis compleja.
- Su ciclo se completa en 10-12 días a 25ºC



Otras ventajas de Drosophila



http://superfly.ucsd.edu/homophila

Table 1 | **Categories of human disease genes well suited to analysis in *Drosophila melanogaster****

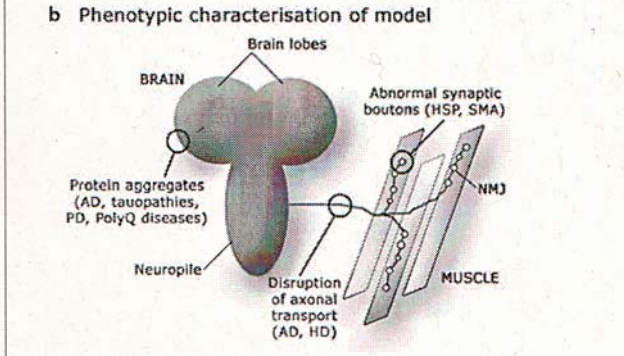
Disease	Human gene symbol	Fly gene symbol	Gene product	References
Dysmorphology				
Sympolydactyly	*HOXD13*‡	*Abd-B*§	Transcription factor	140–142
Single bone in zeugopod	*HOXD3–HOXD13* (heterozygous deletion)	*Abd-B*§	Transcription factor	143,144
Hand-foot-genital syndrome	*HOXA13* or heterozygous *HOXA11–13* deletion	*Abd-B*§	Transcription factor	145–149
Aniridia	*PAX6*	*ey*§, *toy*§	Transcription factor	150–153
Townes-Brocks syndrome	*SALL1*	*salm*§, *sal*§	Transcription factor	154–156
Saethre-Chotzen syndrome	*TWIST1*	*twi*§	Transcription factor	133
Pfieffer syndrome	*FGFR1*, *FGFR2*	*htl*§	RTK	157
Apert syndrome	*FGFR2*	*htl*§	RTK	157
Crouzon syndrome	*FGFR3*	*htl*§	RTK	157
Saethre-Chotzen syndrome-like	*FGFR3*, gain-of-function?	*htl*§	RTK	133
Alagille syndrome	*JAG1*	*Ser*§, *Dl*§	Notch ligand	158
Spondylocostal dysostosis	*DLL3*	*Dl*§	Notch ligand	159
Primary congenital glaucoma	*CYP1B1*	*Cyp18a1*§	Cytochrome P450	108,109
Cardiac disease				
Congenital heart disease	*NKX2-5* *GATA4*	*tin*§ *pnr*§	Transcription factor Transcription factor	160–163 163–165
Holt–Oram syndrome	*TBX5*	*Doc1–Doc3*§	Transcription factor	166–168
DiGeorge syndrome	*TBX1*	*org-1*§, *bi*§	Transcription factor	169
Venous malformations	*TEK*	*htl*§	RTK	170
Neurological				
Spinocerebellar ataxia	*SCA1* (also known as *ATXN1*) *SCA2* (also known as *ATXN2*) *SCA6* (also known as *CACNA1A*) *SCA14* (also known as *PRKCG*) *SCA17* (also known as *TBP*)	*CG4547* *CG5166* *cac*§, *Ca-α1D*§ *inaC*§, *Pkc53E* *Tbp*§	Transcription cofactor? Unknown Ca^{2+} ion channel Ca^{2+}-dependent PKC TATA binding protein	27,30,171
Huntington disease	*HD*	*huntingtin*§	Axonal transport?	30,172–174
Spinal and bulbar muscular atrophy 3	*AR*	*ERR*, *svp*§	Androgen receptor	27,171
Parkinson disease	*PARK2* *PARK5* (also known as *UCHL1*) *PARK7* *NR4A2* *MAPT* *PINK1* *SNCA*	*park*§ *Uch* *dj-1β*, *CG6646* *Hr38*§ *tau*§ *CG4523*§ None known	E3-ubiquitin ligase Ubiquitin pathway Androgen-R regulator? Nuclear hormone receptor Microtubule binding PTEN-induced kinase Dopamine transmission?	49–51 28–30 30,175 176 28–30
Alzheimer disease	*PSEN1*, *PSEN2* *APP*	*Psn*§ *Appl*§	γ-Secretase Signalling, axonal transport?	27–30,66
Fragile X syndrome	*FMR1*	*Fmr1*§	Translational regulator	72,177,178
Angelman syndrome	*UBE3A*	*dube3A*§	E3-ubiquitin ligase	179,180
Cancer				
Tuberous sclerosis	*TSC1*, *TSC2*	*tsc1*§, *tsc2*§	GAP for RHEB in TOR pathway	86,181–184
Endometrial carcinoma	*PTEN*	*Pten*§	Negative regulator PI3K	86,92
No known disease mutations in homologue	*LATS1*	*wts*§ (also known as *lats*)	Cyclin regulation?	94,95
Renal cancer lines	*SAV1*	*sav*§	Cyclin regulation?	96
No known disease mutations in homologue	*MST1*, *MST2* (also known as *STK3*)	*hpo*§	Cyclin regulation?	97
Bladder and colorectal cancer	RAS family genes	*Ras85D*§	RTK signalling	185
No known disease mutations in homologues	*SCRIB*, *LLGL1*, *DLG1*	*scrib*§, *l(2)g*§, *dlg1*§	Cell polarity, metastasis in the presence of RAS-V12	
B-cell leukaemia	*CCND1*	*CycD*§	Cell cycle	93
Melanoma	*CDK4*	*Cdk4*§	Cell cycle	
Retinoblastoma	*RB1*	*Rbf*§, *Rbf2*	Cell cycle	
Hepatocellular carcinoma	*TP53*	*hth*§ ($e<10^{-10}$)	Cell cycle	
Ectodermal dysplasia	*TP73L*	*hth*§ ($e<10^{-7}$)	Cell cycle	

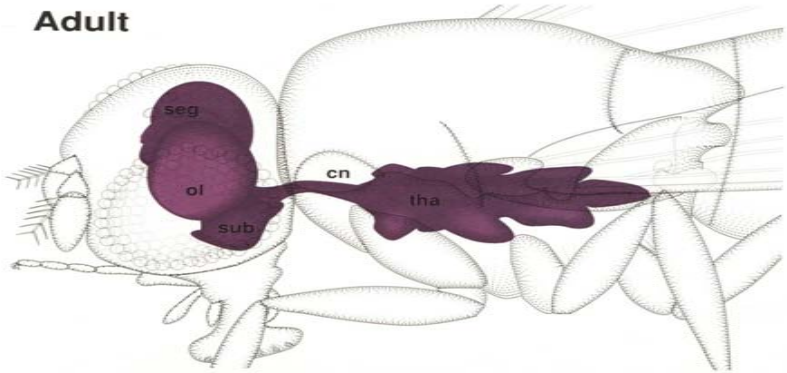
*An extended version of this table with additional disease categories can be found online. All e-values $<10^{-20}$ unless otherwise indicated. ‡Mild heterozygotes and strong null phenotypes. GAP, GTPase-activating protein; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PTEN, phosphatase and tensin homologue; RAS-V12, a constitutively active form of RAS; RHEB, RAS homologue enriched in brain; RTK, receptor tyrosine kinase; TOR, target of rapamycin. §*D. melanogaster* genes for which mutant as well as wild-type alleles have been isolated.

Nature Genetics Reviews, 6 (2005)

Un Sistema Nervioso relativamente complejo

Neurodegenerative Dis, 3 (2006)





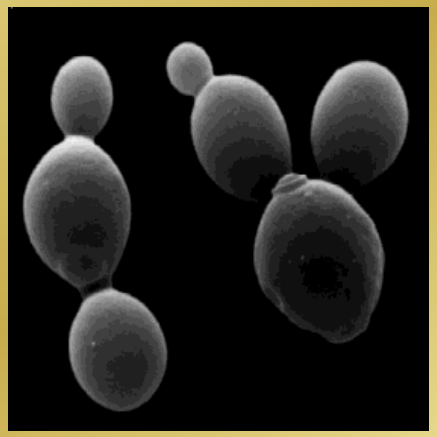
Unlike most other larval organs, the CNS persists into the adult stage. Recent evidence (for review, see Truman 1990; Truman et al., this volume) suggests that most motor neurons and large interneurons of the insect adult nervous system are of embryonic origin. To this set of embryonically born neurons, a large number of neurons are added during larval and early pupal stages. The neuroblasts that generate these postembryonic neurons seem to be the same as those that had produced the larval CNS in the embryo (Prokop and Technau 1991). In the first larval instar, these neuroblasts reappear at the outer surface of the CNS (see L3 on facing page). In some parts of the CNS (e.g., thoracic segments), they closely match the number of neuroblasts present in the embryo (Truman and Bate 1988). In other regions (e.g., posterior abdominal segments), the number of postembryonic neuroblasts is very small. Each neuroblast resumes its proliferatory activity. As before in the embryo, neuroblasts divide in a stem cell mode with a perpendicularly oriented spindle. Their progeny, the presumptive adult neurons, remain undifferentiated until pupal stages. The optic lobe (*ol*), which started out as a small vesicle attached to the basal surface of the early larval brain, proliferates and gives rise to the outer and inner optic anlagen (outer optic anlage, *ooa* on drawing of pupa). These structures are curved epithelial sheets that cap the lateral aspect of the brain hemispheres throughout larval life. The outer optic anlage forms the lamina and part of the medulla; the inner optic anlage gives rise to the remaining part of the medulla, the lobula, and the lobula plate (Hertweck 1931; Hofbauer and Campos-Ortega 1990; see Meinertzhagen and Hanson this volume). The gross anatomy of the CNS changes markedly during late postembryonic development (Hertweck 1931). The larval brain hemispheres (*br*), to which the massive optic lobes and several central brain structures are added, become the supraesophageal ganglion (*seg*) of the adult brain. Another part of the adult brain is the subesophageal ganglion (*sub*). This structure develops from the neuromeres of the three gnathal segments that, in the larva, had formed part of the ventral ganglion (*vg*). The three thoracic neuromeres of the larval ventral nerve cord, which had grown massively by postembryonic neuroblast proliferation, form the major part of the adult thoracico-abdominal ganglion (*tha*). The fused abdominal neuromeres remain small; they form an unpaired "cone" attached to the third thoracic neuromere. (*cn*) Cervical connective.

Volker Hartenstein. Cold Spring Harbor Laboratory Press (1993)

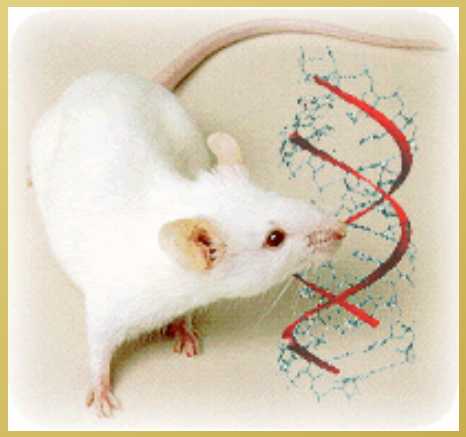
Modelos de enfermedades neurodegenerativas en Drosophila

Enfermedad de Alzheimer
Enfermedad de Parkinson
Enfermedades poli-Q • Enfermedad de Huntington • Ataxia espinocerebelosa (SCA): SCA 1, 2, 3, 6, 7, 17 • Atrofia muscular espinobulbar (SBMA) • Atrofia palidolusiana dentatorubral (DRPLA)
Enfermedades trinucleotidos tipoII • Síndrome del X frágil • Ataxia de Friedreich • Distrofia miotónica

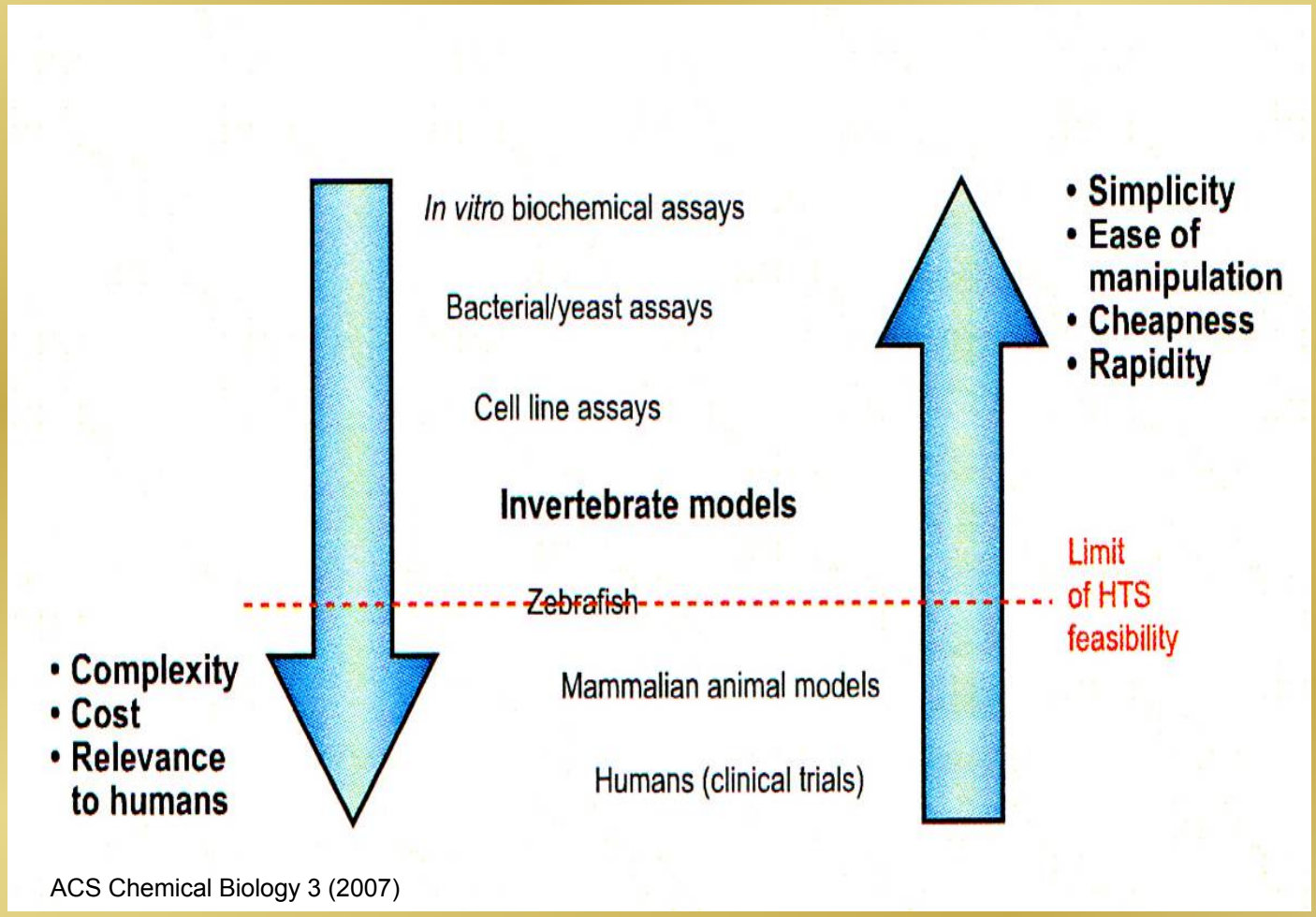
Drosophila: modelo genético sencillo con un nicho intermedio



Funciones autónomas de la célula eucariota

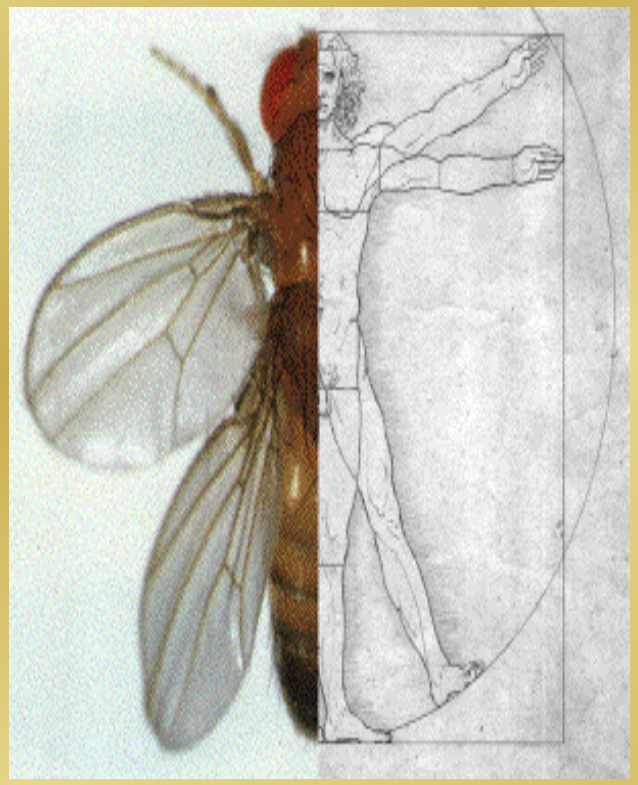


Interacciones entre células, funcionamiento de órganos y sistemas



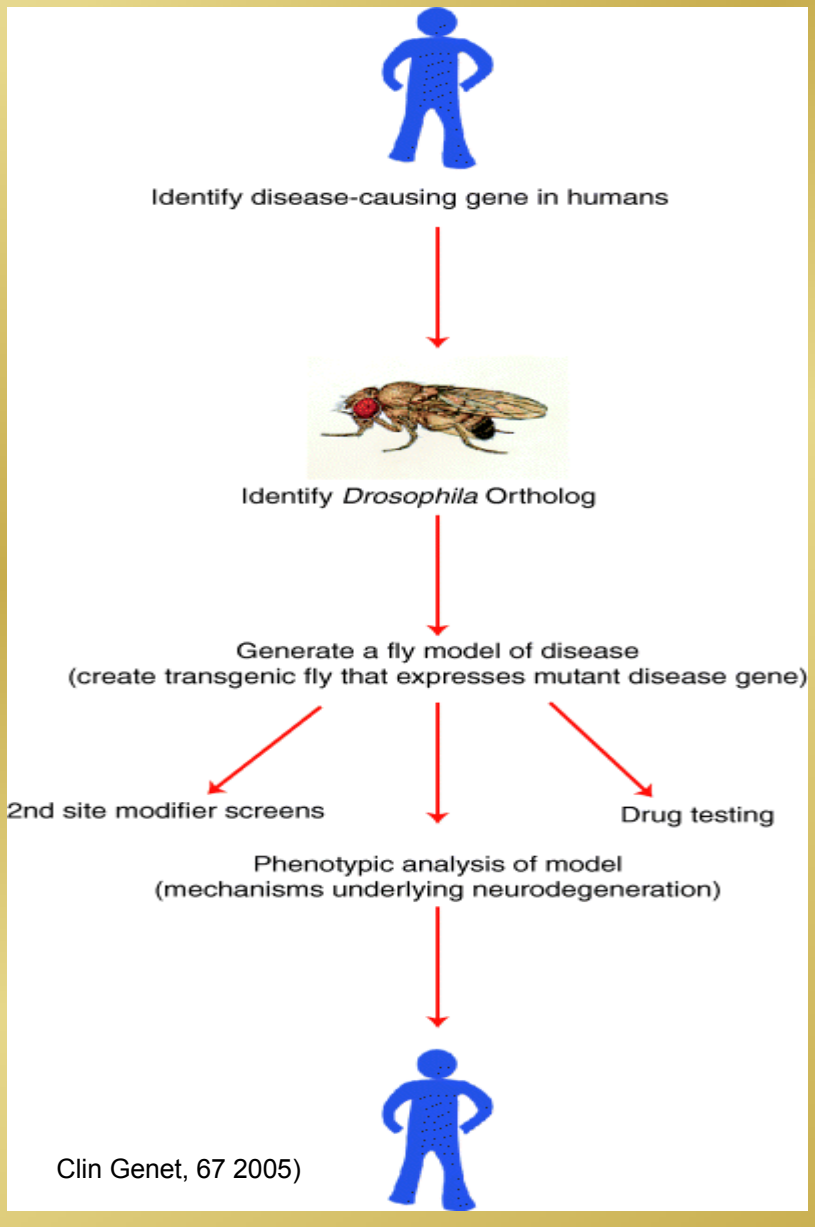
ACS Chemical Biology 3 (2007)

Modelos biomédicos en Drosophila



1. Testar nuevas hipótesis sobre la patología
2. Identificar los genes involucrados en un mismo proceso celular
3. Identificar nuevos fármacos

Obtención del modelo



Mutaciones patológicas:

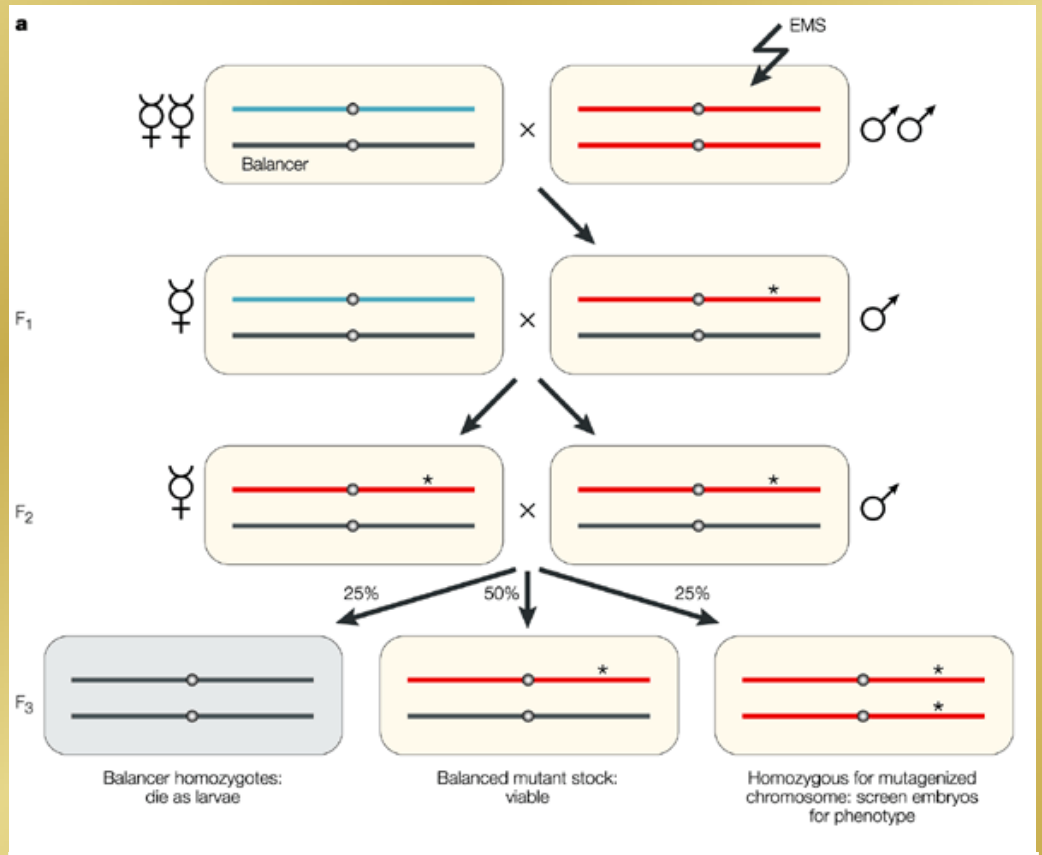
Pérdida de función

- mutagénesis química
- mutagénesis mediante elementos P
- RNAi

Ganancia de función

- Sobre-expresión del gen endógeno
- Expresión del alelo normal del gen humano
- Expresión del alelo patológico del gen humano
- Generación de mutaciones patológicas en el gen endógeno

Mutagénesis química



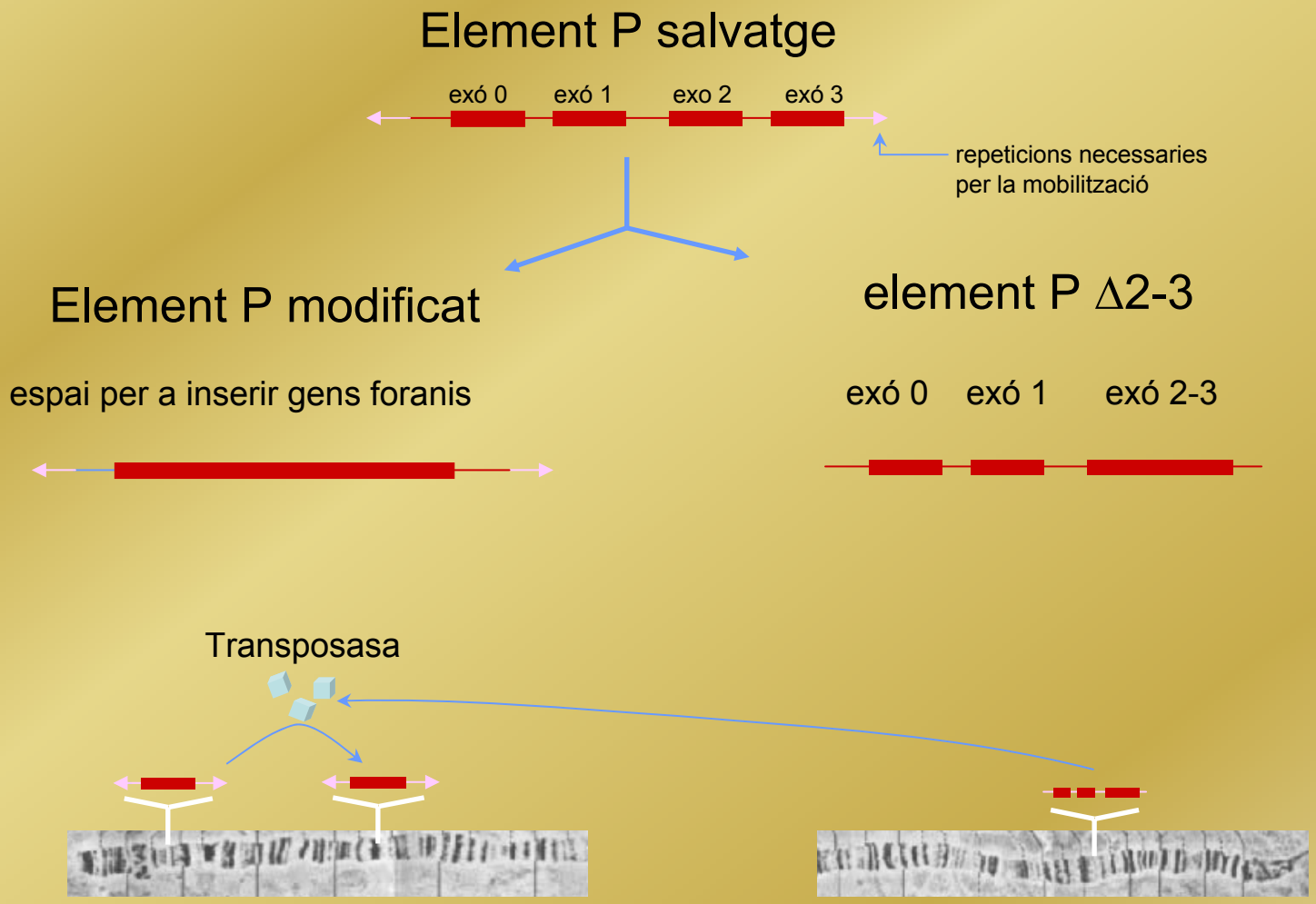
Localización de la mutación

Incorporación a un mapa de ligamiento de SNPs

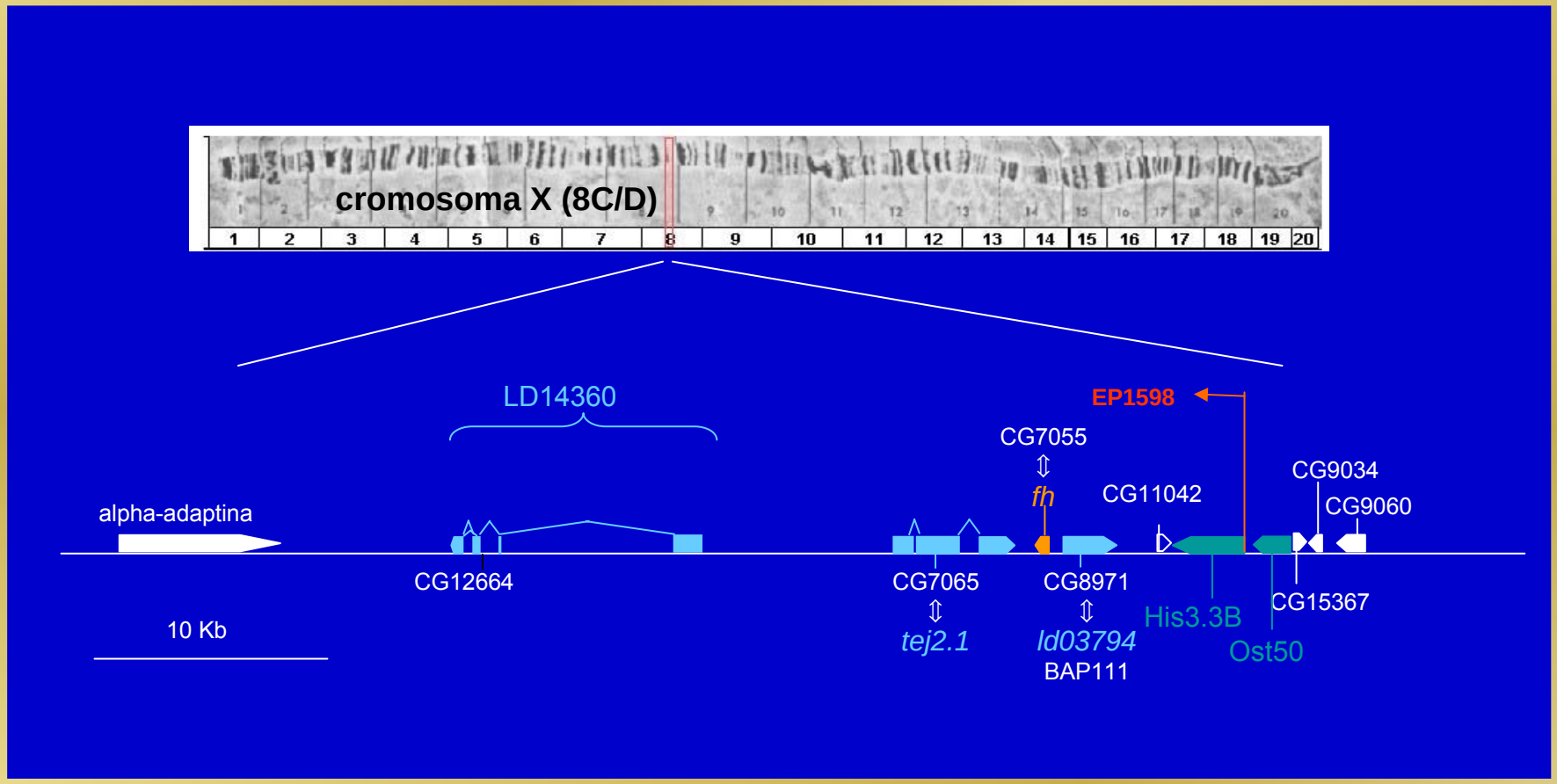
PCR del gen. Secuenciación de los amplificados

Nature Genetics Reviews, 3 (2002)

Mutagénesis insercional con elementos P



Transposición local de P



Diseño de los cruces

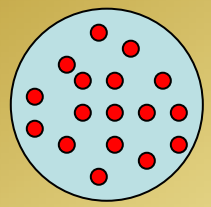
Parentales

♀♀ $\frac{w\ EP(w^+)}{w\ EP(w^+)}\ \frac{+}{+}$ x $\frac{w}{Y}\ \frac{TMS(Sb)\Delta 2\text{-}3}{Dr}$ ♂♂

Sb, ♀

F1

♀ $\frac{w\ EP(w^+)}{w}\ \frac{+}{TMS(Sb)\ \Delta 2\text{-}3}$ x $\frac{FM7c}{Y}\ \frac{+}{+}$ ♂



No Sb, w^+, ♀

F2

♀ $\frac{w\ EP(w^+)}{FM7c}\ \frac{+}{+}$ ♂♂ $\frac{FM7c}{Y}\ \frac{+}{+}$

Líneas establecidas

Resultados movilización de P en 8CD

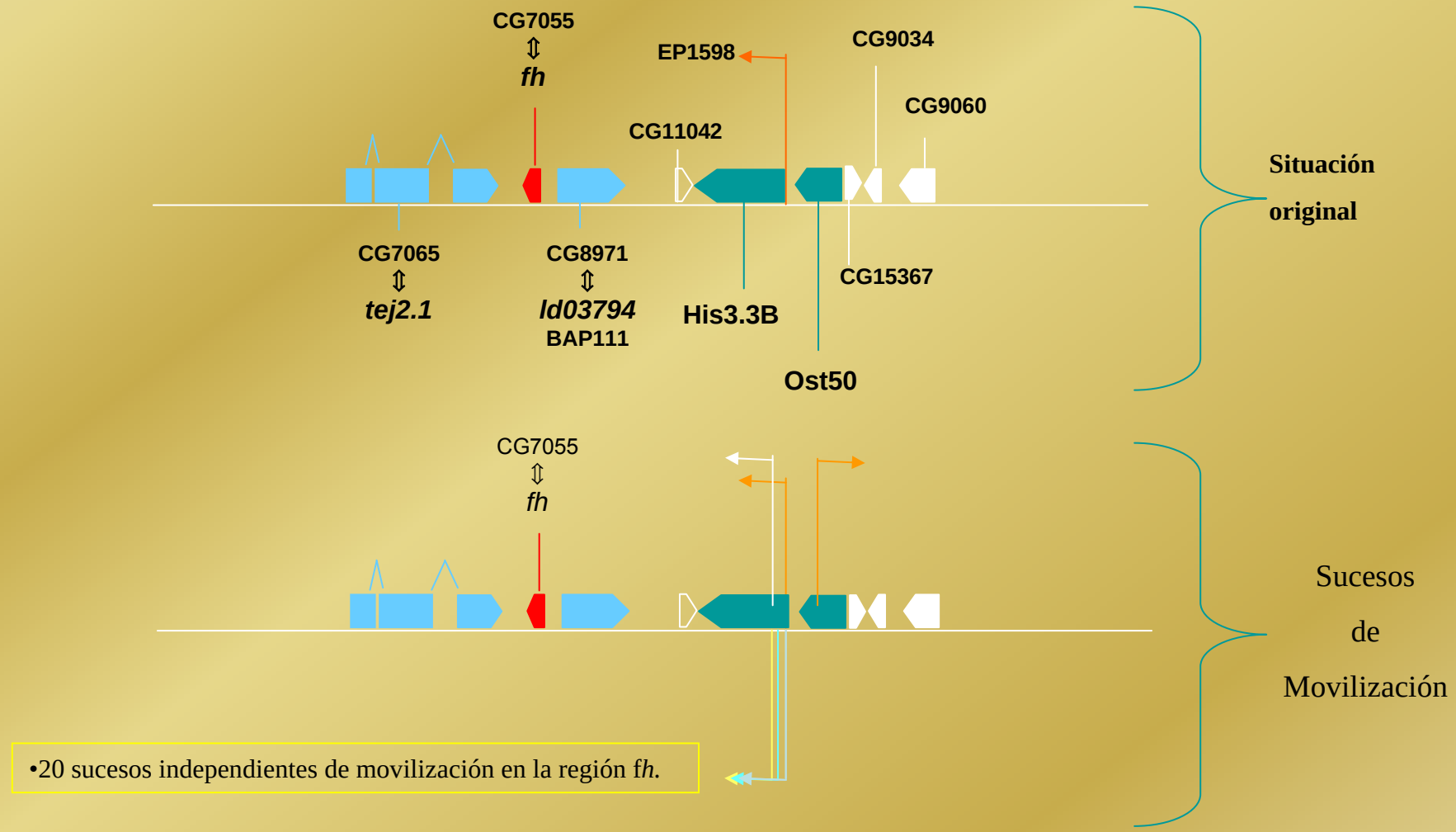


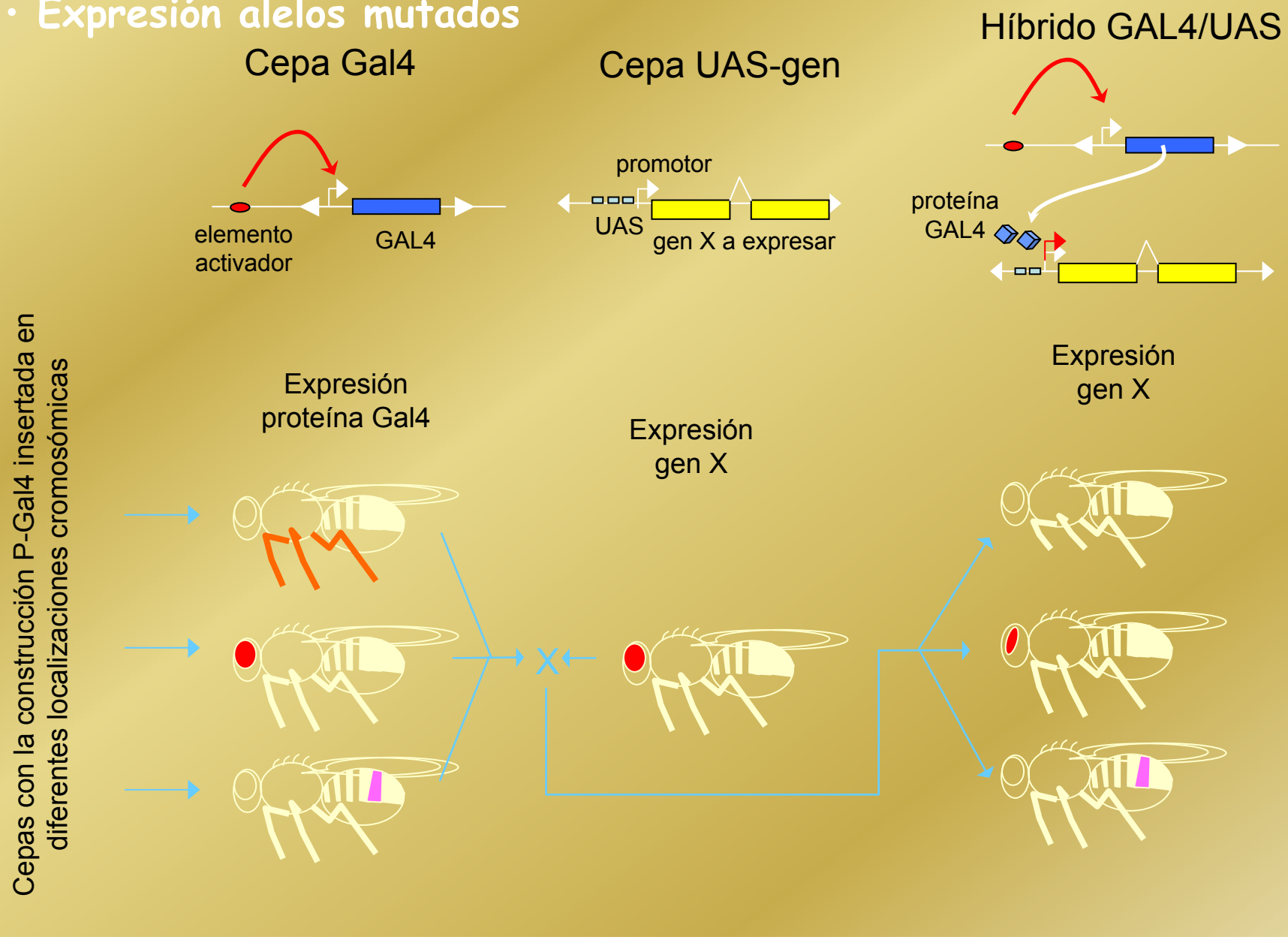
Table 2. Examples of well-characterised neurodegenerative mutants isolated using forward genetics in *Drosophila*

Screen design	Isolated mutant/gene	Characterised mutant phenotype	Ref.
P element insertion mutagenesis for life-span-reducing mutants	*bubblegum* mutant mapped to the VLCFA acyl-coenzyme A synthetase-like gene	Adult neurodegeneration; elevated levels of VLCFAs as seen in the human disease adrenoleukodystrophy	118
EMS-induced mutagenesis for life-span-reducing mutants; isolated mutants were then examined for histological neuropathology	*spongecake* mutant; gene not mapped	Brain degeneration characterised by regionally specific, membrane-bound vacuoles, similar to those seen in spongiform degenerations (e.g. Creutzfeldt-Jakob disease)	119
	eggroll mutant; gene not mapped	Brain degeneration characterised by dense, multilamellated structures resembling those found in lipid storage diseases (e.g. Tay-Sachs disease)	
EMS-induced mutagenesis followed by eye-specific expression of Flp recombinase to generate patches of homozygous mutant tissue; mutants isolated on whether the eyes develop substantial black degenerate tissue	*burned* mutant mapped to the *cpb* locus encoding the β-subunit of the F actin capping protein; *scorched* mutant mapped to the *cpa* locus encoding the α-subunit of the F actin capping protein	Mutant neuronal tissues characterised by accumulation of F actin consistent with the known function of the isolated genes in capping actin filaments and arresting their growth	120
P element insertion mutagenesis for abnormal development of the peripheral nervous system	*benchwarmer* mutant mapped to the *spinster* gene which is predicted to encode a lysosomal sugar carrier	Abnormal lysosomal carbohydrate storage, synaptic defects, subsequent progressive neuronal degeneration and enhanced tau-mediated toxicity suggesting aberrant lysosomal function and defects in endocytic membrane trafficking	121, 122
EMS-induced mutagenesis for temperature-sensitive paralytic mutants	*vacuous* mutant; gene not mapped	Locomotor defects in both larvae and adults, neuronal hyperactivity and extensive age-dependent neurodegeneration throughout the CNS	123
	Two mutants mapped to the same gene, *ATPa*, which encodes the α-subunit (catalytic) of the Na^+/K^+-ATPase	Behavioural abnormalities, reduced life-span, severe neuronal hyperexcitability and extensive, age-dependent neurodegeneration suggesting that maintenance of neuronal viability is dependent on normal sodium pump activity	124
P element insertion mutagenesis for mutants with histological adult brain abnormalities	*sniffer* mutant mapped to the *sniffer* gene encoding a short-chain dehydrogenase/reductase enzyme	Reduced life-span, a progressive sluggish walking phenotype and age-related neurodegeneration, possibly the result of oxidative stress	125, 126
EMS-induced mutagenesis for mutants exhibiting adult brain defects as determined by histology	*swiss cheese* mutant mapped to the *swiss cheese* gene encoding a protein sharing homology with vertebrate neuropathy target esterase	Progressive neurodegeneration, glial hyperwrapping, neuronal apoptosis and reduced life-span	127, 128
EMS- and *P*-element-insertion-induced mutagenesis for mutants exhibiting adult structural brain defects as determined by histology	*vacuolar peduncle* mutant mapped to the *RasGAP* gene which encodes Ras GTPase-activating protein	Age-related brain degeneration, possibly the result of deregulation of the EGFR/Ras signalling pathway	129
Mutagenesis via *P* element enhancer insertions for gene products exhibiting a gain-of-function by causing neural cell loss	*Effecter for neuronal death and degeneration 2* mutant mapped to the *Sec61a* gene which encodes for an endoplasmic-reticulum-associated protein translocon	Neuronal cell death accompanied by the accumulation of ubiquitinated proteins	130

VLCFA = Very-long-chain fatty acid; EMS = ethyl methane sulphonate; EGFR = epidermal growth factor receptor.

Sistema UAS-GAL4:

- Sobre-expresión del alelo normal
- Expresión alelos mutados



Modelos de enfermedades neurodegenerativas

Enfermedad	**Generación del modelo** Expresión de las secuencias humanas
Enfermedad de Alzheimer	- péptidos $A\beta_{40}$ y $A\beta_{42}$. - gen APP - genes APP y β-secretasa - Gen dPS portador de mutaciones patológicas
Enfermedad de Huntington	- fragmento aminoterminal de Htt con Q75, Q93, Q120 o Q128.
Ataxia de Friedreich	- Disminución de la expresión del gen endógeno (fh)

ENFERMEDAD DE ALZHEIMER

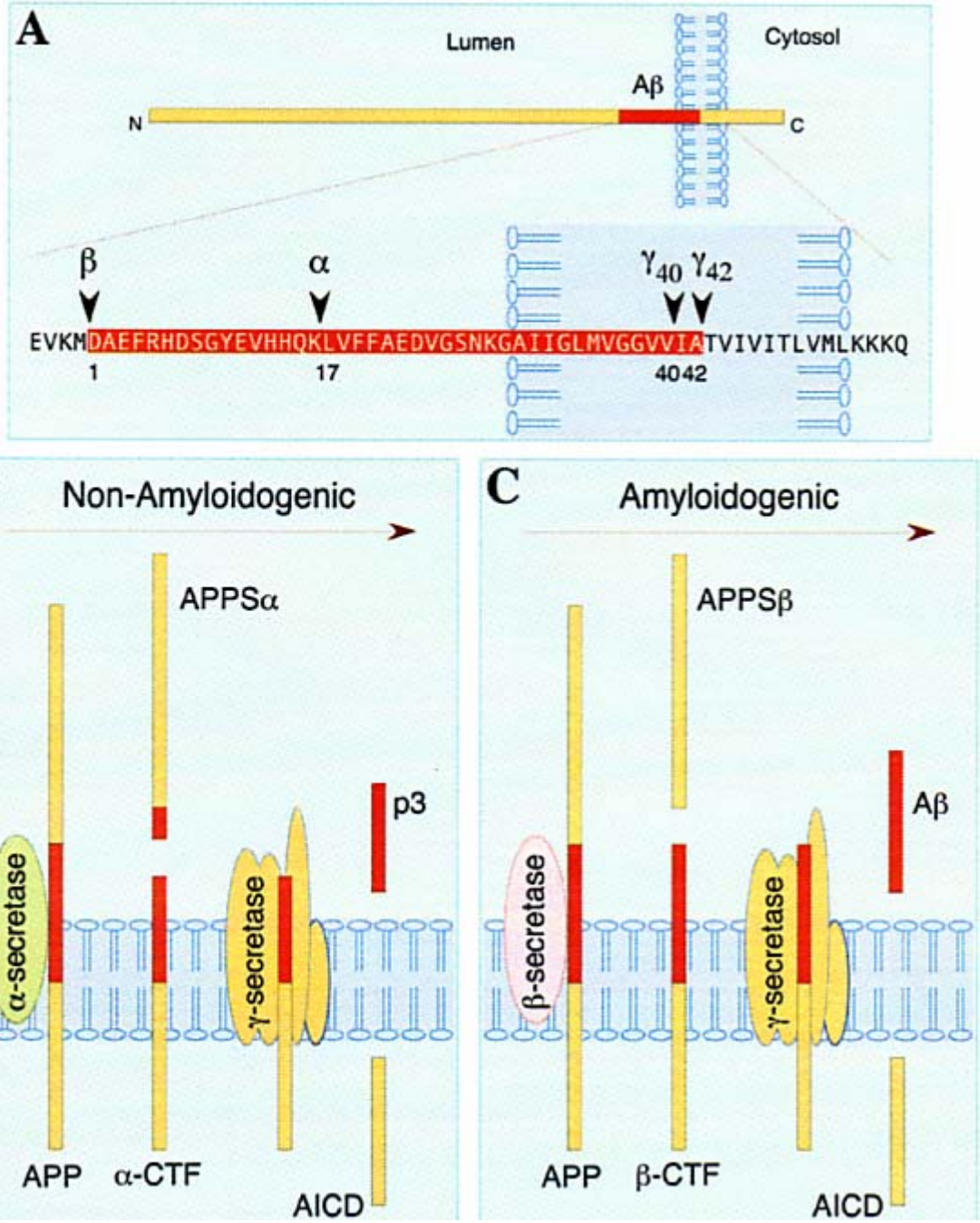


Figure 1. Proteolytic processing of APP. (A) Schematic structure of APP is shown with Aβ domain shaded in red and enlarged. The sites of cleavage by α-, β-, and γ-secretases are indicated along with Aβ numbering from the N-terminus of Aβ (Asp1). (B) Nonamyloidogenic processing of APP refers to sequential processing of APP by membrane-bound α- and γ-secretases. α-Secretase cleaves within the Aβ domain, thus precluding generation of intact Aβ peptide. The fates of N-terminally truncated Aβ (p3) and APP intracellular domain (AICD) is not fully resolved. (C) Amyloidogenic processing of APP is carried out by sequential action of membrane-bound β- and γ-secretases.

Modelos de enfermedades neurodegenerativas

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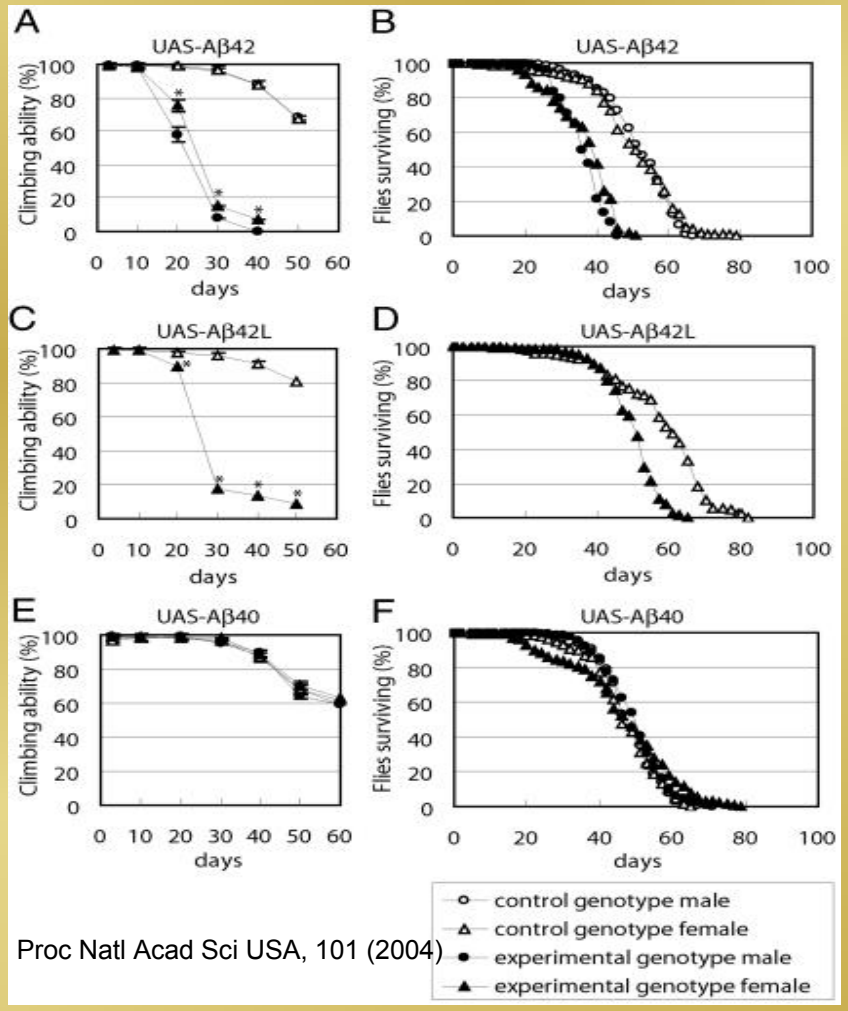
Ataxia de Friedreich	- Disminución de la expresión del gen endógeno (fh)

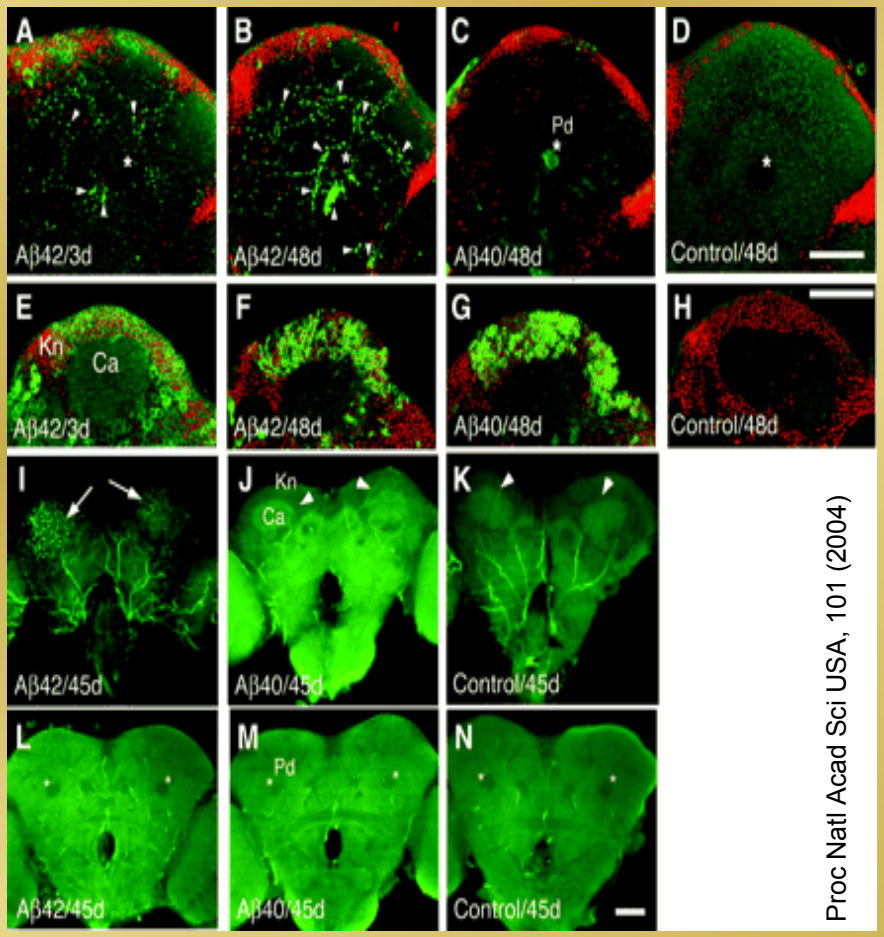
Modelo de E. Alzheimer en Drosophila - expresión del péptido Aβ



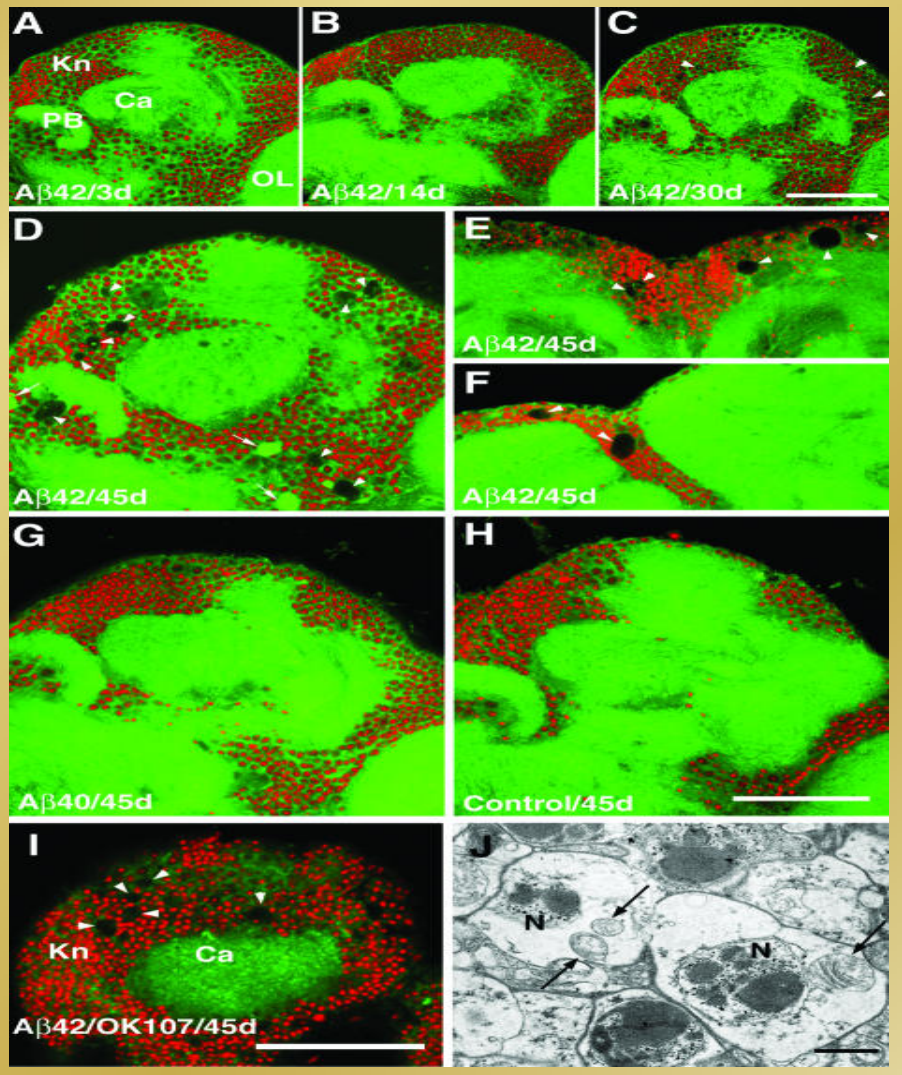
Proc Natl Acad Sci USA, 101 (2004)

- Reducción de la capacidad de escalada
- Reducción de la esperanza de vida
- Formación de depósitos Aβ
- Pérdida de neuronas
- Alteraciones progresivas

Modelo de E. Alzheimer en Drosophila



Proc Natl Acad Sci USA, 101 (2004)

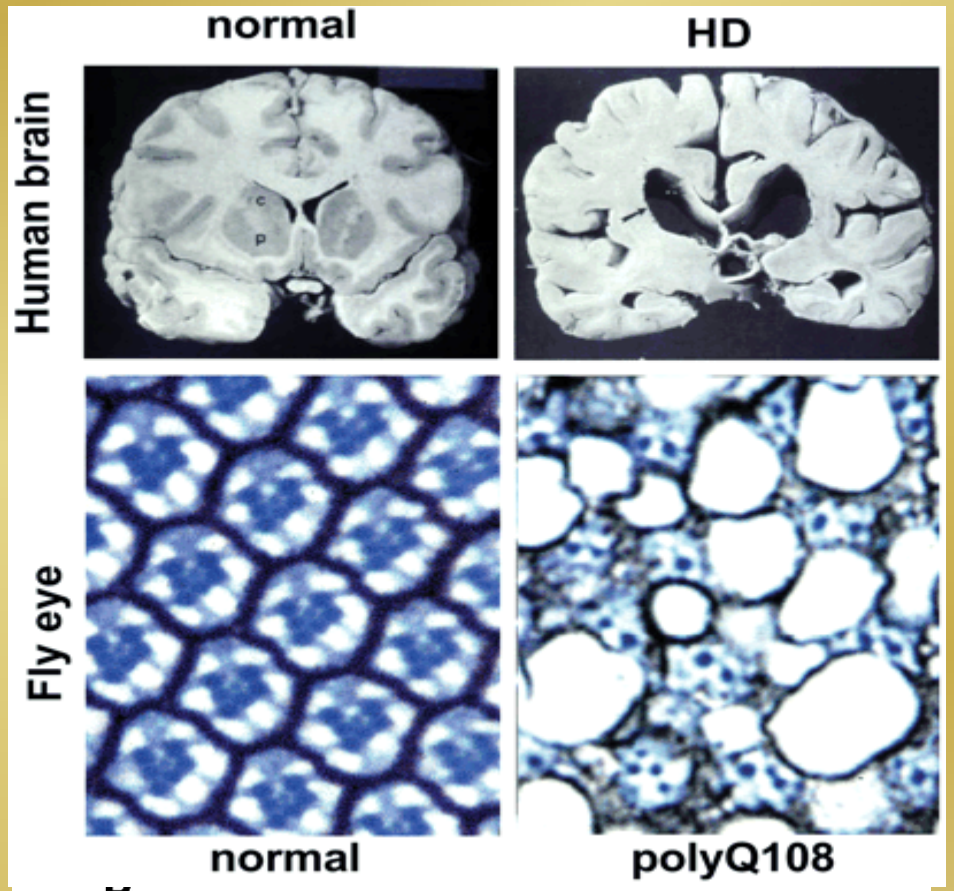


Modelo de E. Huntington en Drosophila

→ fragmento aminoterminal de Htt con Q75, Q93, Q120 o Q128

La patología es:

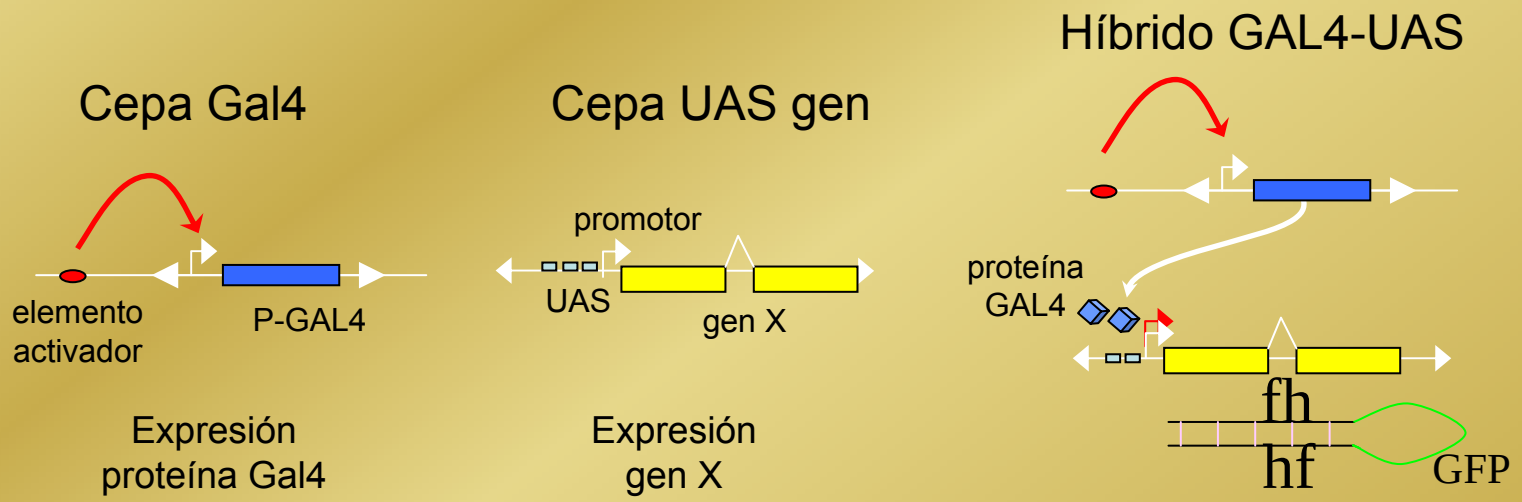
- Dependiente de la longitud poli-Q
- Inicio tardío
- Progresiva
- Pérdida de neuronas
- Causa la muerte



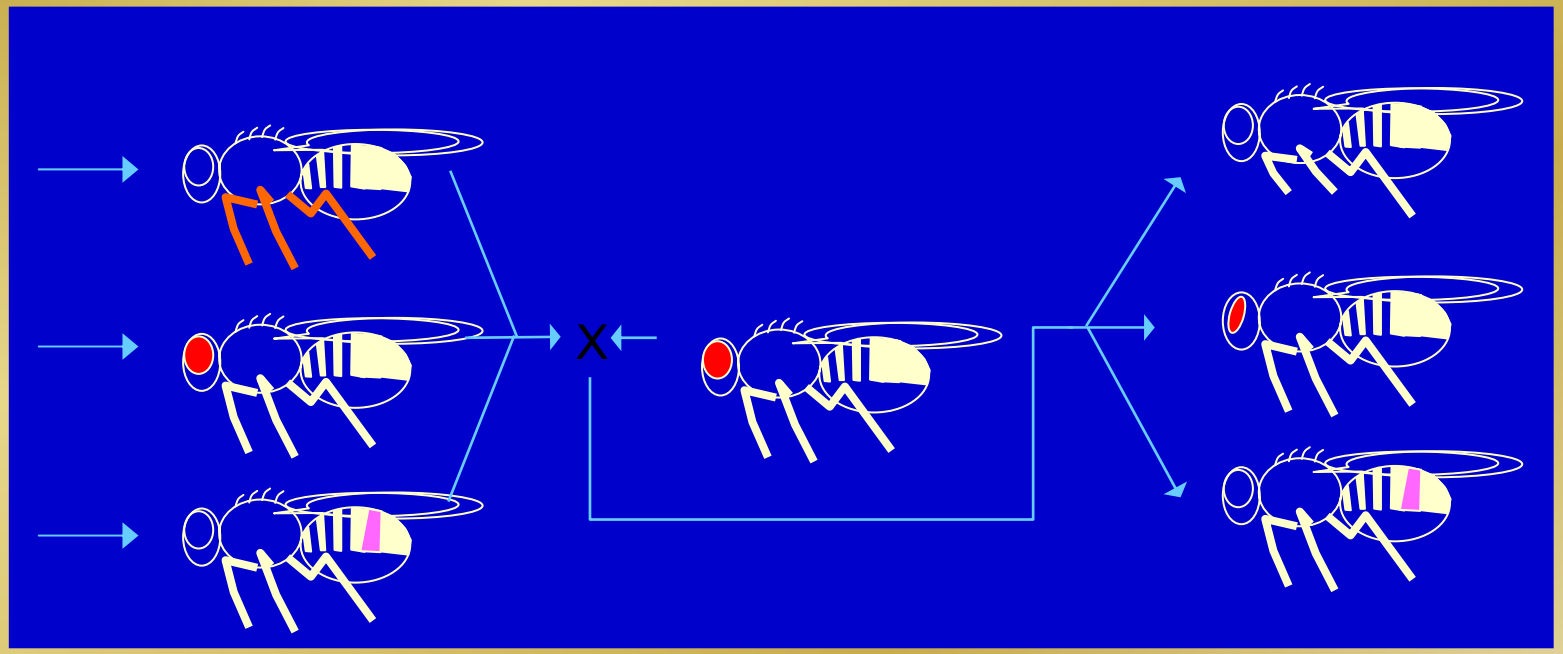
Hum Mol Genet., 12 (2003

Sistema UAS-GAL4

- **Disminución expresión génica por RNAi**



Cepas con la construcción P-Gal4 insertada en diferentes localizaciones cromosómicas



Modelo de ataxia de Friedreich en Drosophila

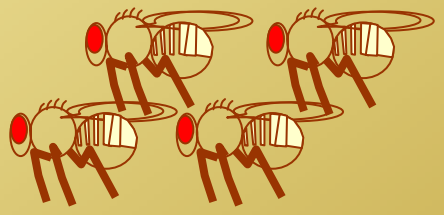
- Reducción sistémica de frataxina en el adulto

Actin-Gal4

X

UAS-*fh*IR

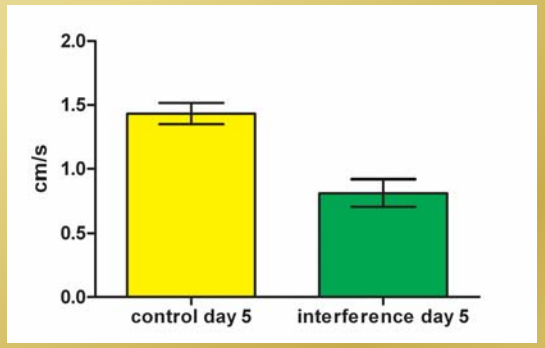
F_1

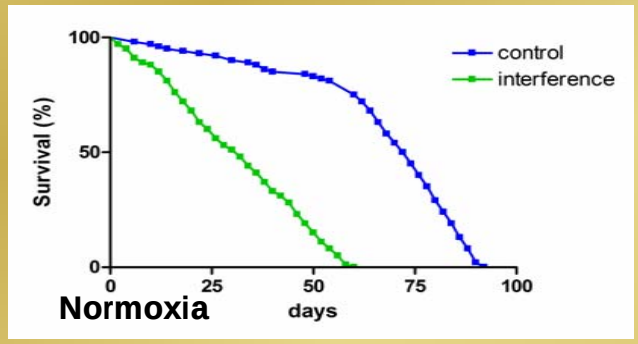


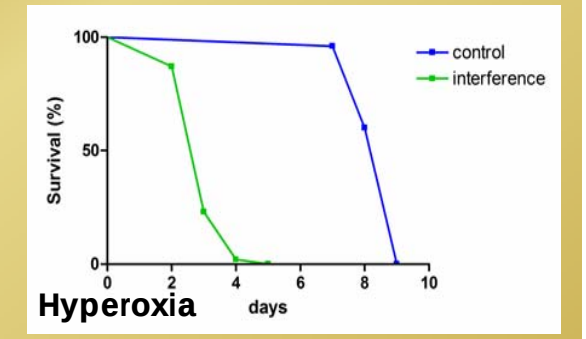
mRNA *fh:* 30%

- supervivencia reducida
- disminución escalada
- sensibilidad frente al estrés oxidativo

• Reducción del 60% vida media
•Reducción del 32% vida máxima

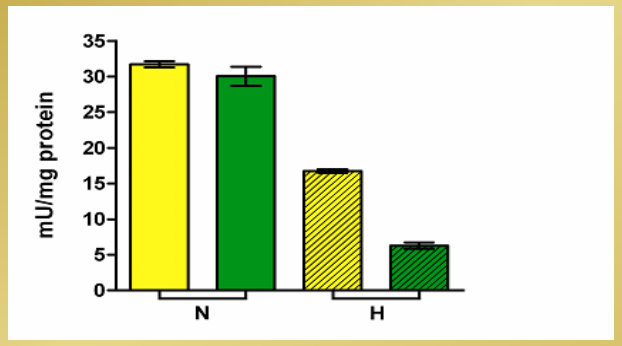


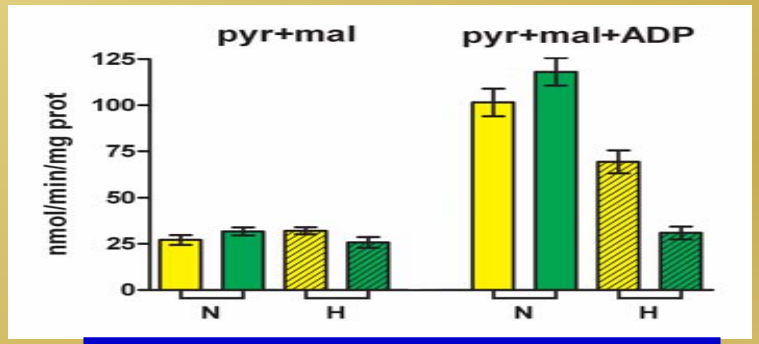




• Reducción del 45% en la capacidad de escalada

• Reducción del 75% de la vida media
• Reducción del 45% de la vida máxima



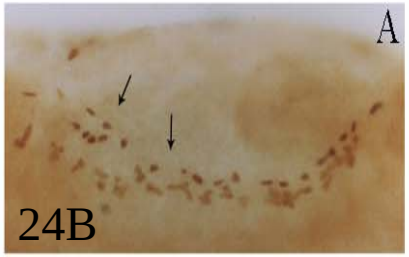


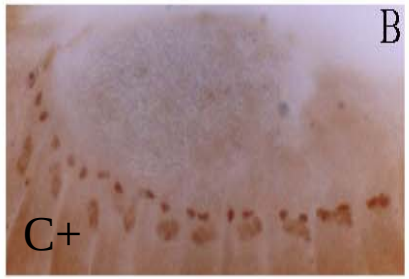
• Mayor inactivación de aconitasa en hyperoxia

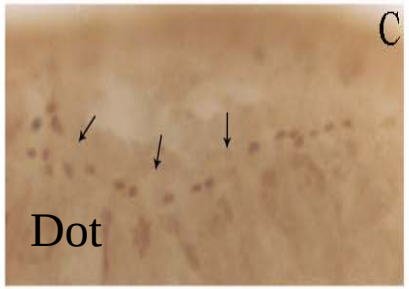
Reducción respiración:
• Controles: reducción del 37%
• Mutantes: reducción del 73%

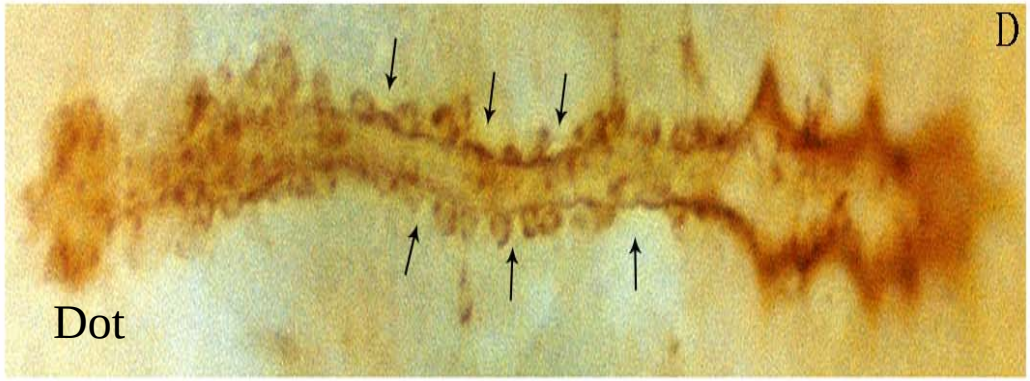
Anti-eve

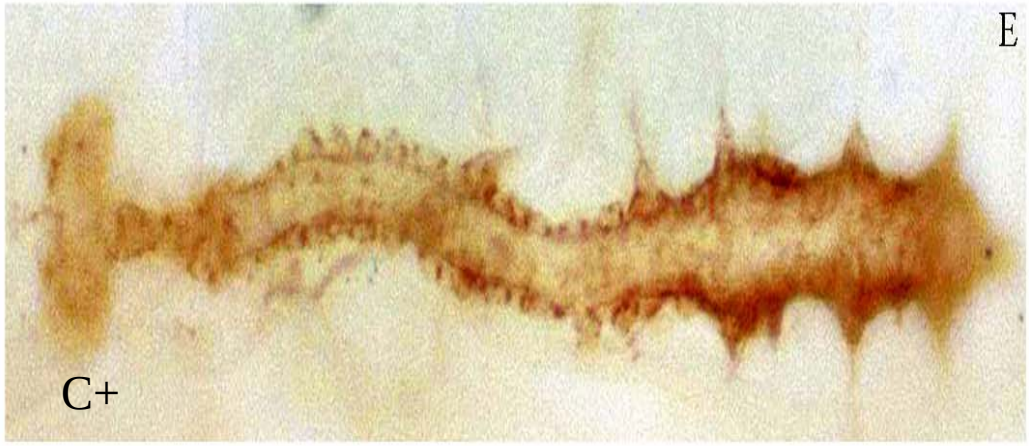
EC11





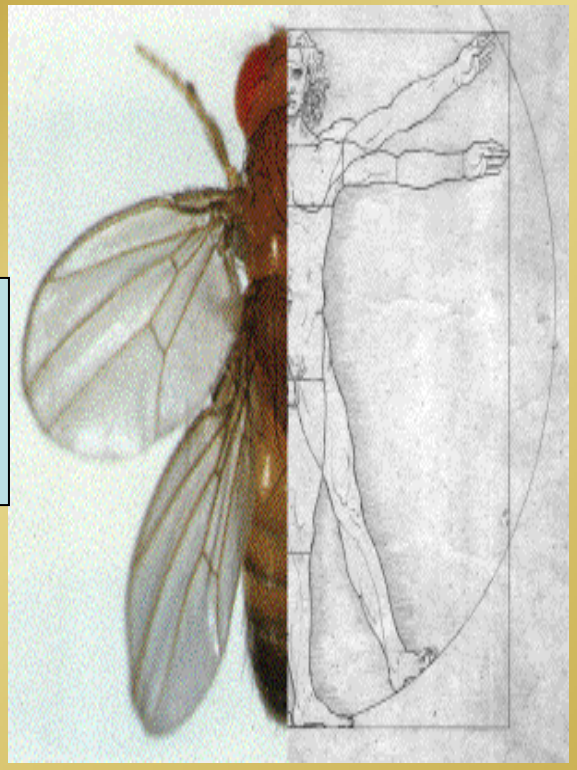






Explotación del modelo

Identificación de genes modificadores mediante rastreos genéticos



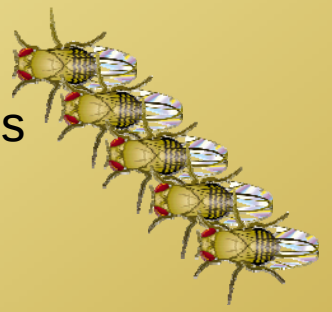
Rastreo de fármacos mediante cribado de compuestos a gran escala

Rastreo de genes modificadores

UAS-gen / + ; Neur-GAL4 / +

X

P-element insertion stocks



P / balancer ; + / +

UAS-gen / balancer ; Neur-GAL4 / +

UAS-gen / P ; Neur-GAL4 / +

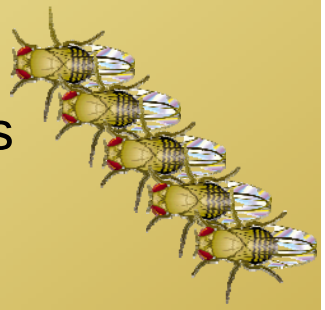
Fenotipo mutante modificado por la reducción de la expresión del gen X

Rastreo de genes modificadores

UAS-gen ; Neur-GAL4
+ +

X

P-element insertion stocks

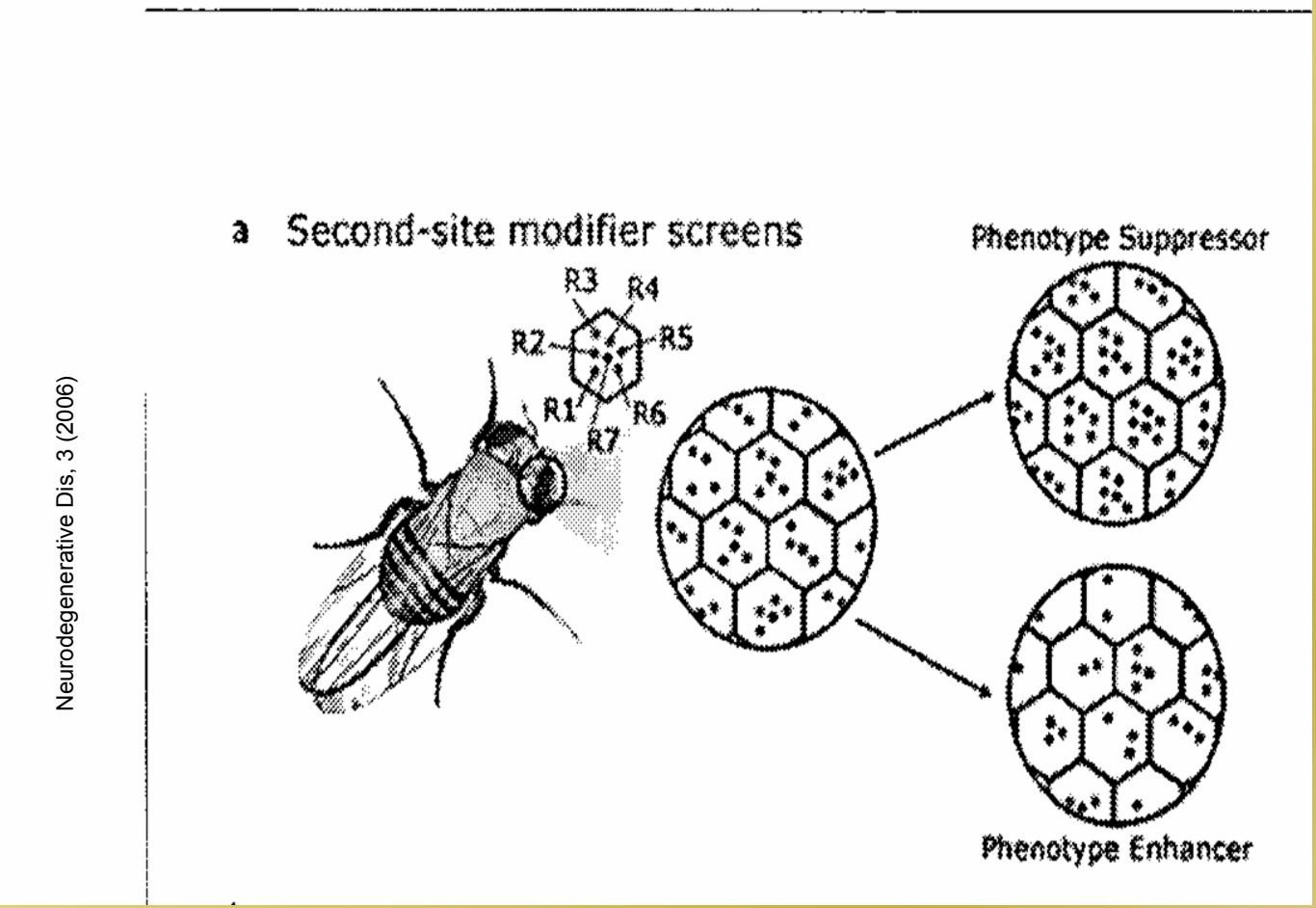


EP ; +
+ +

UAS-gen ; Neur-GAL4
EP +

Fenotipo mutante modificado por la expresion del gen X

Ojo de Drosophila: la estructura preferida en los rastreos genéticos



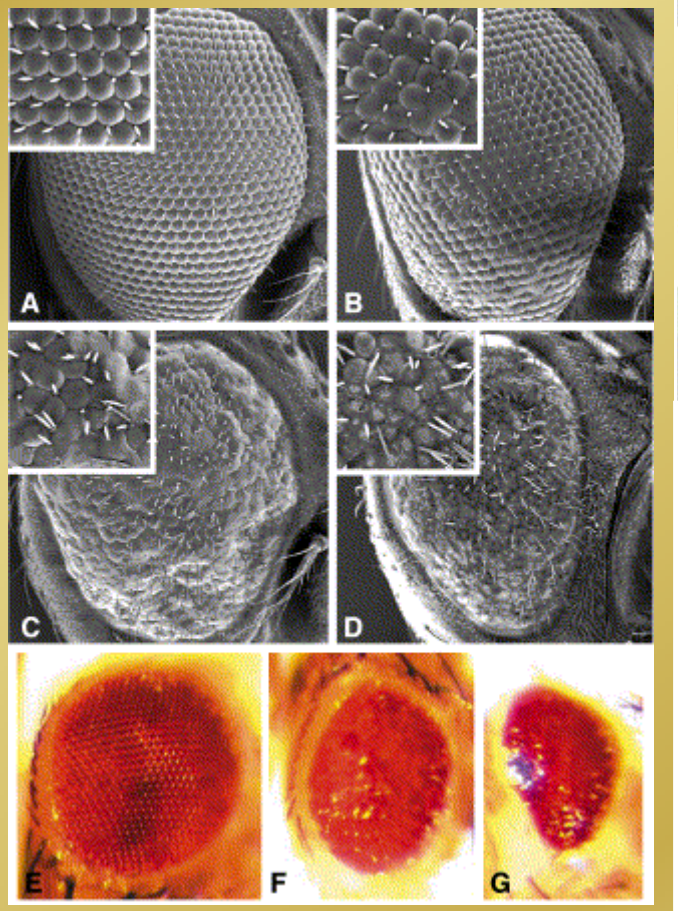
Enfermedad de Alzheimer

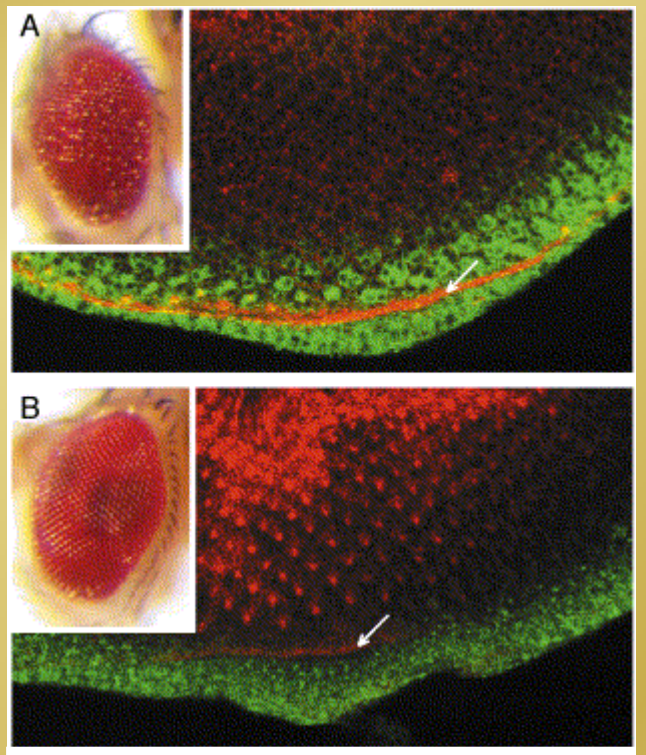
ey-$A\beta_{42}$ X EP (2000)

↓

$\frac{\text{UAS-}\ A\beta_{42}}{\text{EP}}$; $\frac{\text{ey-GAL4}}{+}$

EP(3)3549





EP(3)3549

↓ neprilisina

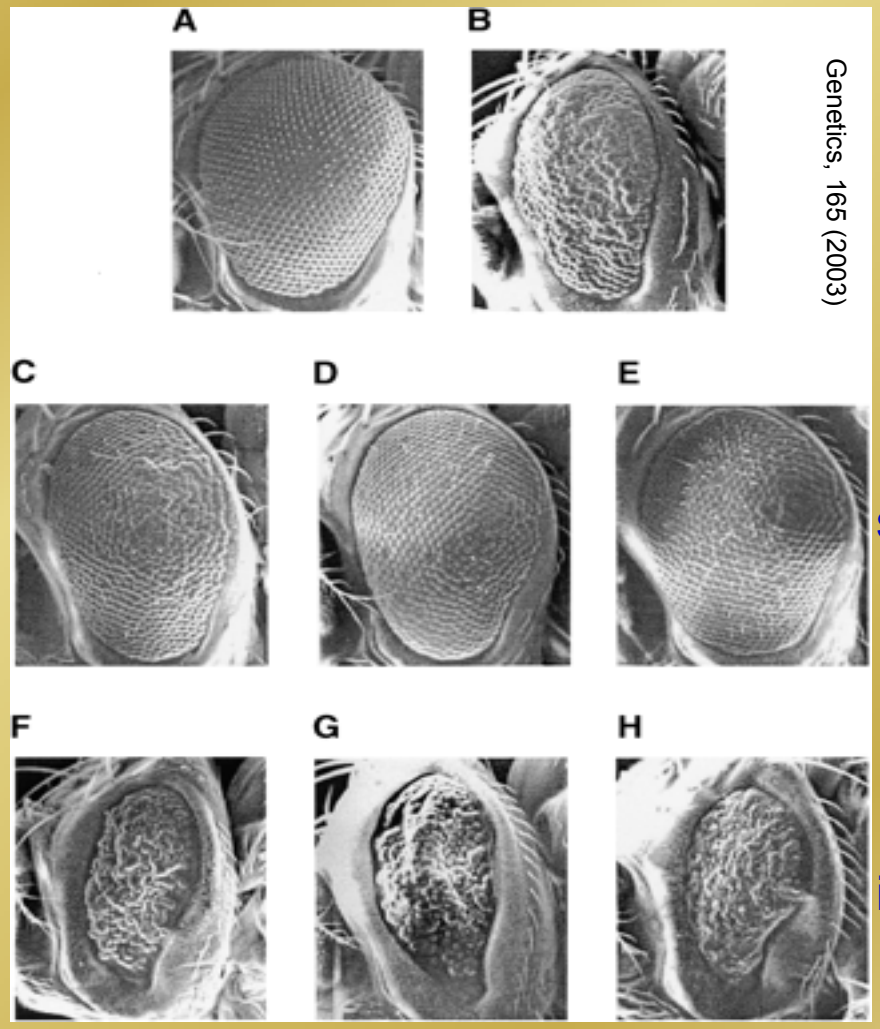
Mol.Cell.Neurosci., 26 (2004)

Enfermedad de Alzheimer

GMR-tau X EP (2276)

UAS- tau ; GMR-GAL4
EP +

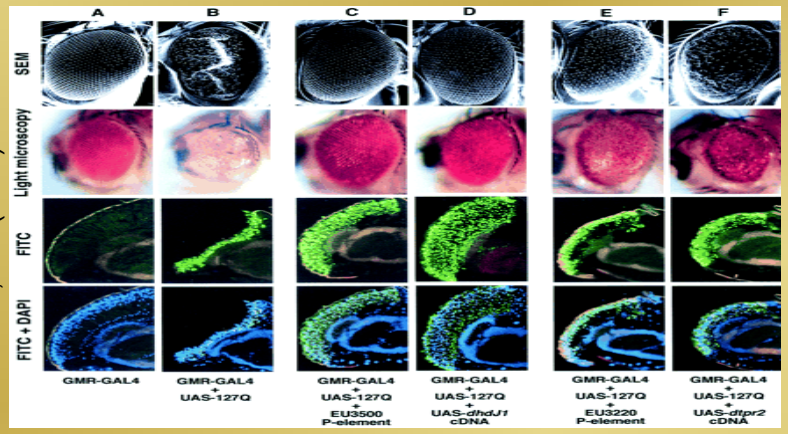
EPs
- Quinasas
- Fosfatasas



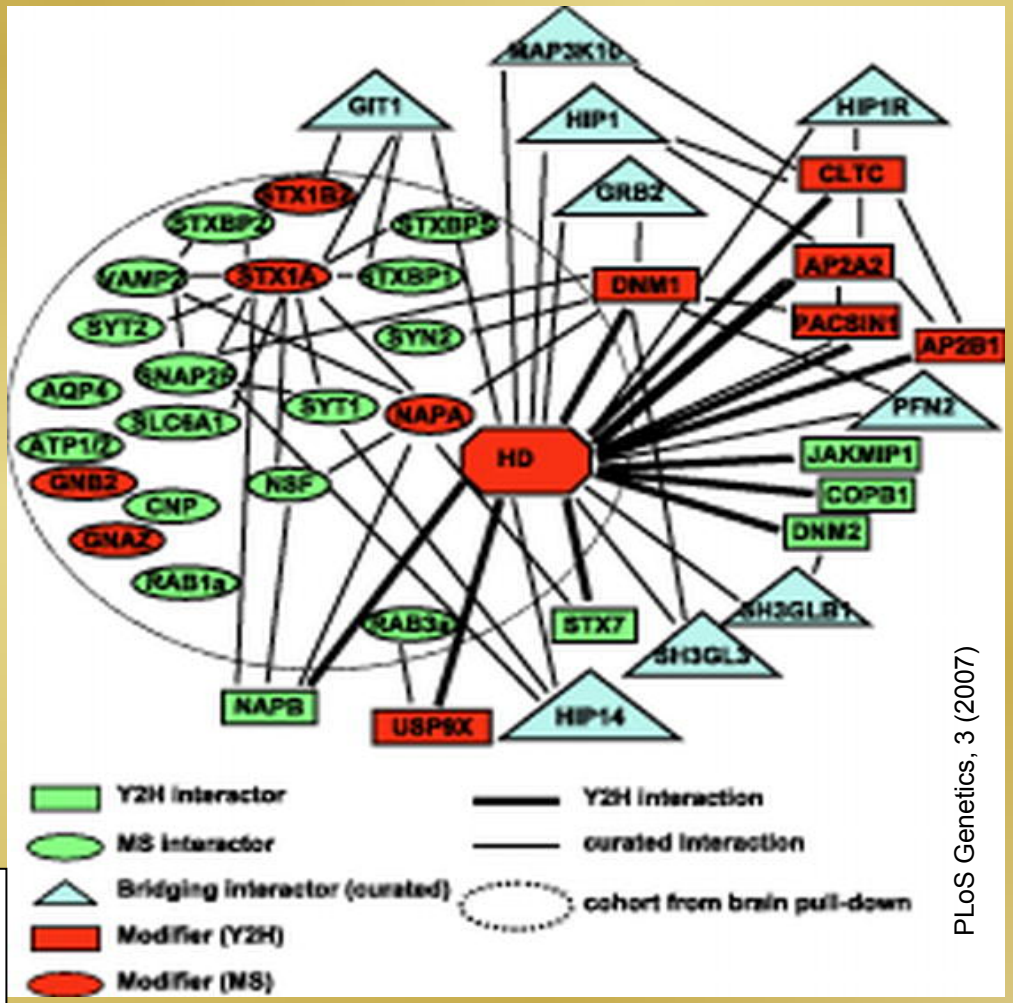
supresores

intensificadores

Enfermedad de Huntington



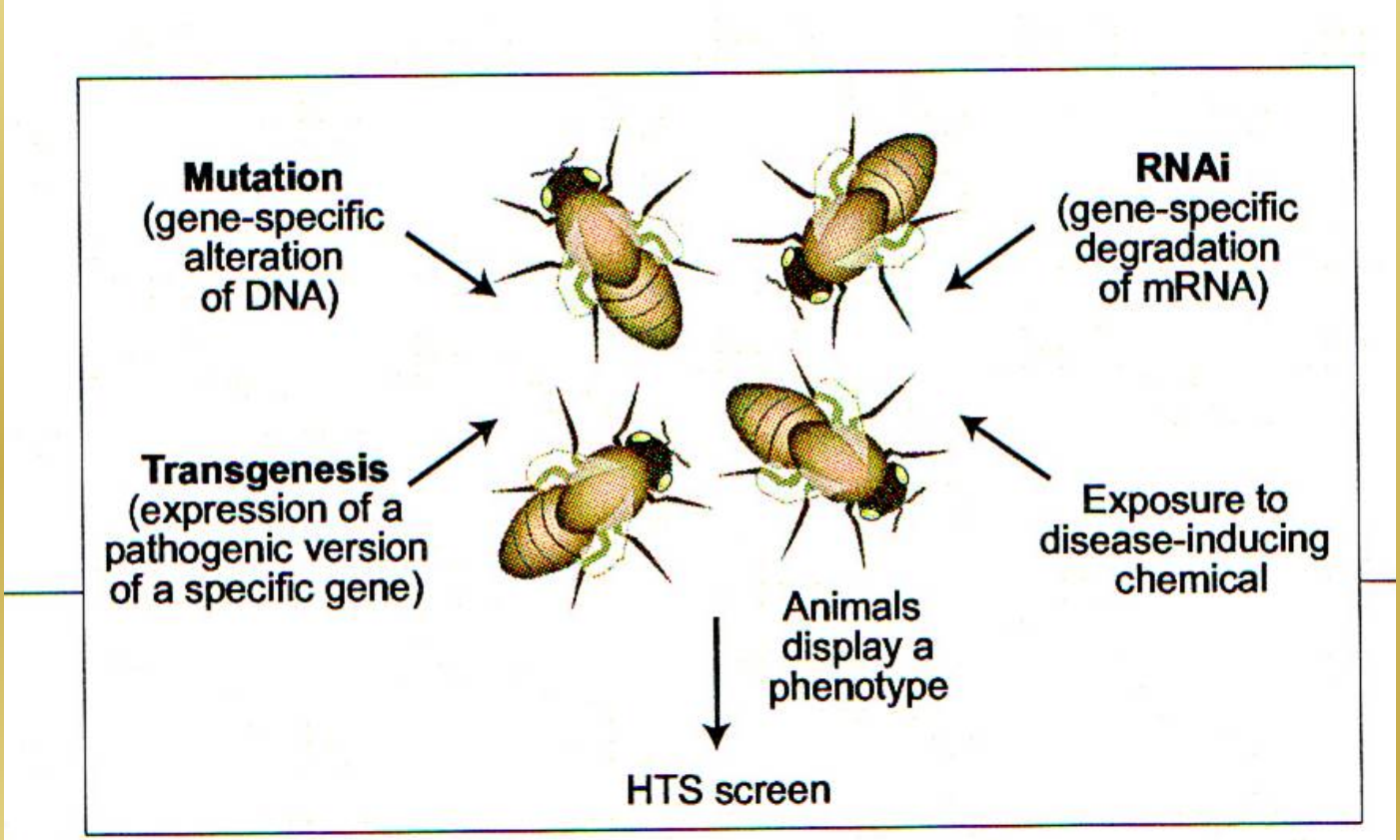
Science, 287 (2000)



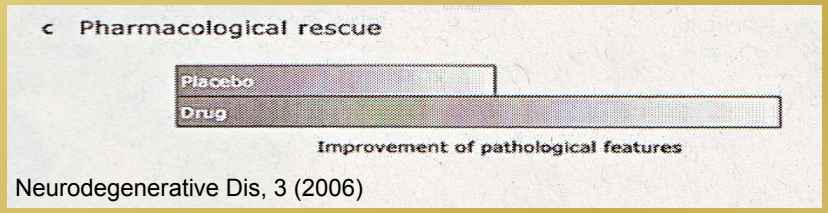
PLoS Genetics, 3 (2007)

- Organización citoesqueleto
- Transducción de señales
- Transmisión sináptica
- Proteolisis
- Regulación transcripción y traducción

Rastreos farmacológicos



Ségalat, ACS Chemical Biology 3 (2007)



Neurodegenerative Dis, 3 (2006)

Table 2. Concordance of drug efficacy in flies and mammals

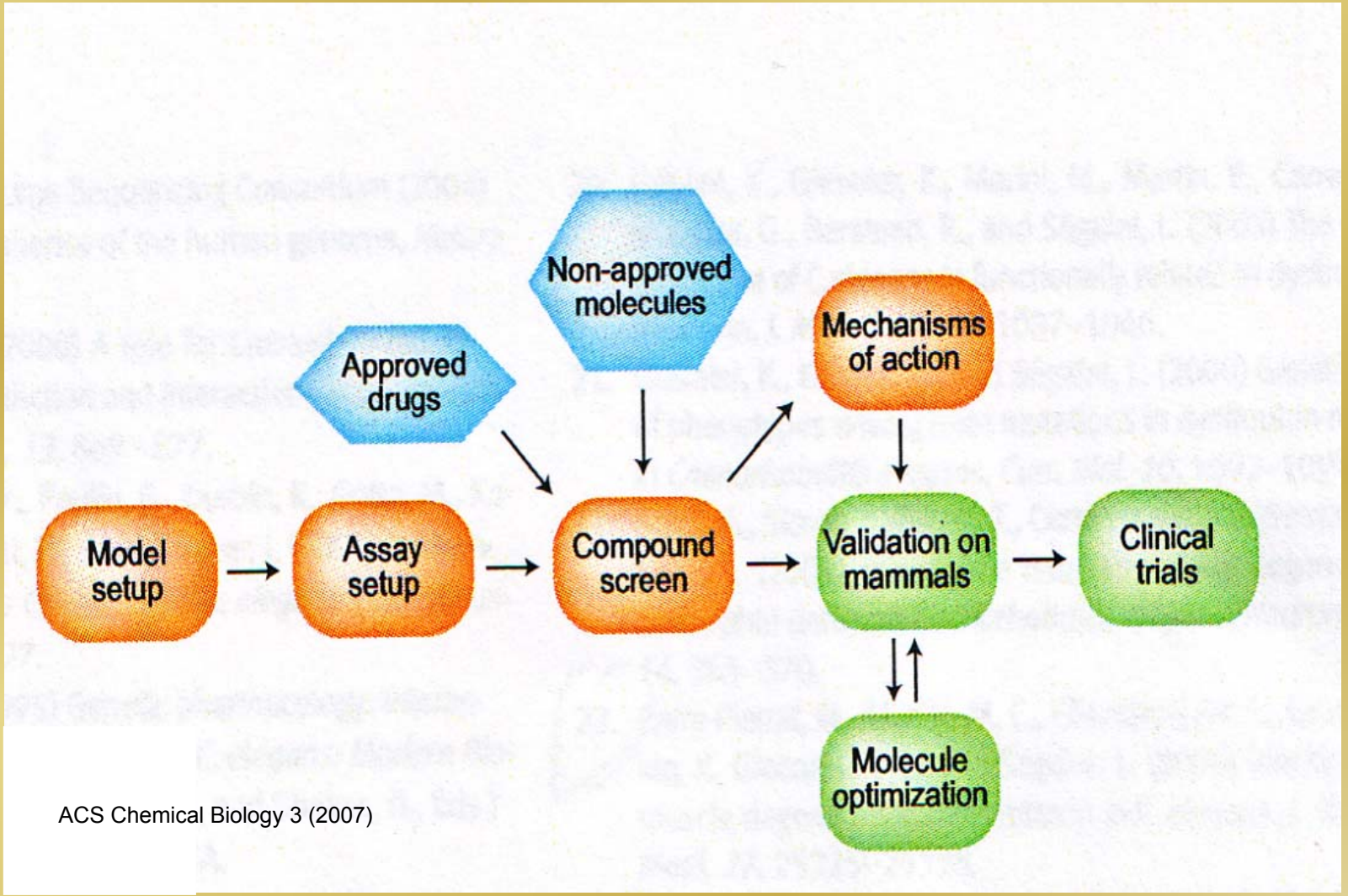
Disease(s)	Drug	Target(s)	Rescue in fly	Rescue in mouse/man	References
HD	SAHA, butyrate	HDAC inhibitor	Y	Y	(44,56,57)
HD-polyQ	Cystamine*	Transglutaminase	Y	Y	(61,63,64)
HD-polyQ	Congo red	Aggregates	Y	Y in brain slices and mice	(60–62)
Parkinson's	Geldanamycin**	Hsp90/Hsp70	Y	Y mammalian cells	(72,77)
Parkinson's	L-DOPA, and dopamine agonists	Dopaminergic function	Y	Y	(73)
ALD	Lorenzo's oil	Restore VLCFA levels to normal	Y	Y	(49)

*While first tested as a potential therapeutic for HD due to its function as a transglutaminase inhibitor, cellular processes targeted by cystamine include inhibition of caspase activity and increased glutathione production.[63–65]

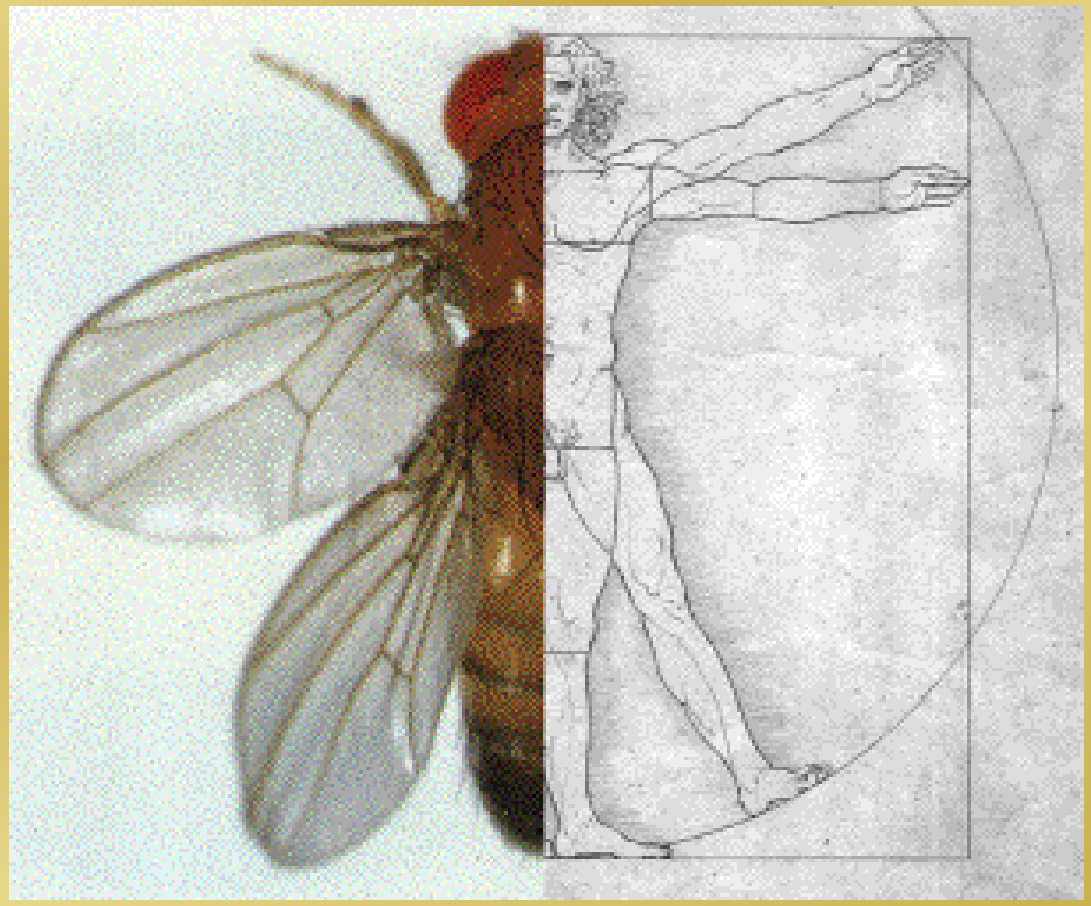
**Geldanamycin has been shown to mount a stress response and improve α-synuclein toxicity in *Drosophila*[33] and to reduce aggregation in mammalian cells expressing mutant Htt.[77] ALD, adrenoleukodystrophy; VLCFA, very long chain fatty acids.

BioEssays, 26 (2004)

Papel de los modelos invertebrados en los tratamientos farmacológicos



ACS Chemical Biology 3 (2007)



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