Cellular and molecular biology of chemoreceptors in *Drosophila*

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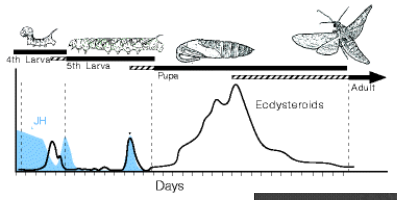
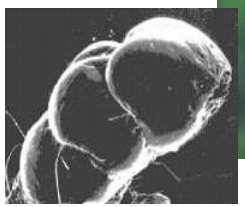
Why using *Drosophila*?

- INRA:
  - SPE: find molecules which can be used to protect plants against pest insects
  - Human and Animal Physiology: use *Drosophila* as a model organism for comparative studies
  - Insect model
- Example:
  - Phytoecdysteroids

Phytoecdysteroids



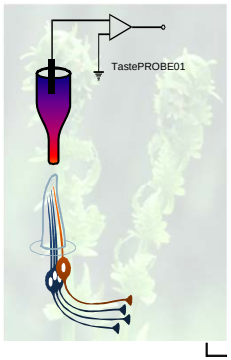
Adapted from Truman J.W., 1992



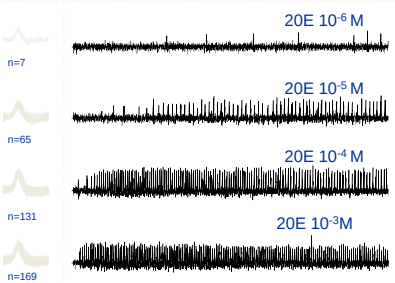
Fall armyworm on *Ajuga remora* (Isao Kubo)

Fougère

Lepidoptera larvae « taste » PEs

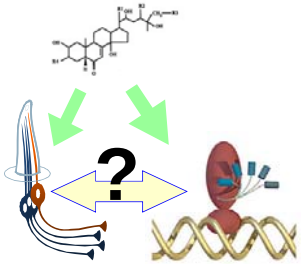


Ostrinia nubilalis L5 - Lateral styloconica



Duration spikes = 6 ms vertical bar = 1 mV horizontal bar= 300 ms

Taste *vs* hormonal receptors?



- Summary
  - 20E + nuclear receptor (Ecr-USP) → express genes
  - 20E + taste receptor → action potentials and feeding inhibition
- Relation?
- To answer this question, we need to work on a model insect

Current projects

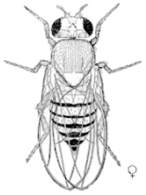
- Habituation to bitter substances in *Drosophila* (Marie-Jeanne Sellier, PhD)
- Selection on food enriched with caffeine or starch (Alexandra Guigue, PhD)
- Taste discrimination learning in *Drosophila* (Marie-Ange Chabaud, Post-doc)
- Aversive and appetitive learning in larval Lepidoptera (Ali Salloum, PhD)
- Olfaction in larval lepidoptera (Kacem Rharrabe, Post-doc & Erwan Poivet, PhD)
- Pathogens sensing in *Drosophila* (Aya Yanagawa, Post-doc)
- Detection of L-canavanine (coll. Y. Grau Montpellier, Moutaz Ali Agha, PhD)

- ANR INSAVEL (Toulouse, Dijon)
- ANR ADAPANTHROP (Gif, Versailles, Lyon)
- Collab. PICASSO (Azucena Gonzalez, Madrrid)

Drosophila = model

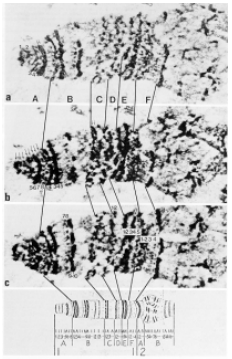
- Genetics genetics!
- Specific advantages:
  - Short cycle
  - Banks of mutants and transformed flies
  - Several tools for genetics and molecular biology
  - Transgenesis
- Genome completely sequenced & DNA chips

Genetics : Morgan



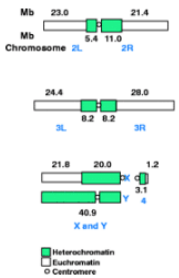
THE PHYSICAL BASIS OF HEREDITY
Thomas Hunt Morgan
Philadelphia: J.B. Lippincott Company
1919

Interesting insect for lab studies



- Short cycle: 10 days
- Polytenic chromosomes (maps)
- Mutagenesis
  - X rays
  - Chemical substances (EMS ethyl methane sulfonate)
  - Transposable P elements

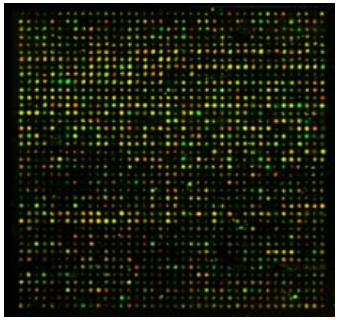
Sequencing achieved



- Celera Genomics + public laboratories
- 180 Mb estimated: 120 Mb euchromatine, 60 Mb non clonable chromatine
- 13061 annotated genes

Adams *et al. Science* (2000) 287:2185-2195

DNA Chips



- DNA Chips available (Affymetrix) with the whole genome
- Specialized DNA chips
- Ex: follow the expression of genes during development

White *et al. Science*, **286**: 2179-2184, 1999
Univ. Yale et Univ. Berkeley
Drosophila genome project

Embryogenesis

	Stage	Time	Developmental events
	1- 4	0:00 - 2:10 h	Cleavage
	5	2:10 - 2:50 h	Blastoderm
	6 - 7	2:50 - 3:10 h	Gastrulation
	8 - 11	3:10 - 7:20 h	Germ band elongation
	12 - 13	7:20 - 10:20 h	Germ band retraction
	14 - 15	10:20 - 13:00 h	Head involution and dorsal closure
	16 - 17	13:00 - 22:00 h	Differentiation

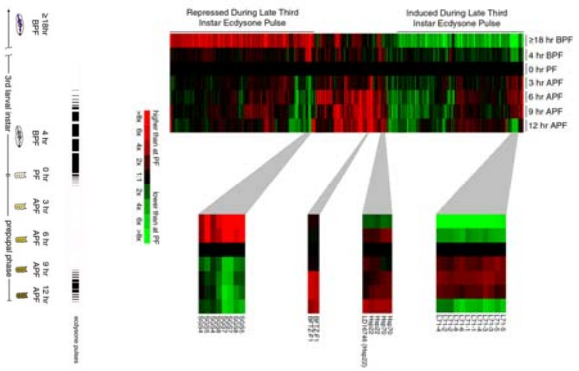
The embryonic development of *Drosophila melanogaster* has been subdivided into 17 stages by Volker Hartenstein and José Campos-Ortega. Staging according to these authors has become a general reference in Drosophila research.

! **Stade 1-4= syncitium**

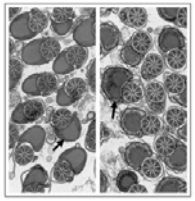
Modified after **Volker Hartenstein** (1993). Atlas of *Drosophila* Development, p 52, Cold Spring Harbor Laboratory Press

http://flyserver.uni-muenster.de/

Expression of genes / development



Model for human diseases (1)



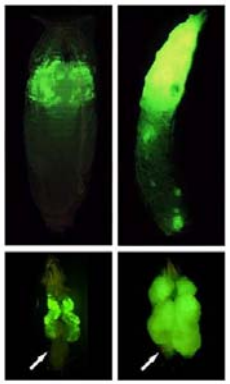
- **Phenotypic analysis of the Drosophila homolog of Parkin - a gene linked to Parkinson's disease.**

  Electron micrograph showing a cross section through tails of mature spermatids from wild type (left) and parkin mutants (right). Loss of parkin function causes mitochondrial dysfunction, shown here by the disruption of the mitochondrial derivative, the Nebernkern (arrows).

  This evidence links a genetic mutation to mitochondrial dysfunction, previously biochemically implicated in the etiology of Parkinson's disease.

  Greene et al., 2003. *PNAS*, 100: 4078-4083.

Model for human diseases (2)



- **Metastatic behavior in *Drosophila*.**

  GFP-labeled tumors made specifically in the eye through expression of oncogenic Ras do not form secondary growths (left top panel) and do not invade adjacent tissues such as the larval central nervous system (arrow, left bottom panel). Inactivation of cell polarity genes such as *scribble* causes the appearance of secondary growths (right top panel) as well as the invasion of cells into the central nervous system (arrow, right bottom panel). As neither oncogenic Ras nor scribble inactivation can cause this phenotype on their own, both alterations cooperate to promote metastatic behavior. This genetic model for metastasis allows for the systematic discovery of genes and cellular processes that may play a role in human malignant cancers.

  R.A. Pagliarini and T. Xu. A Genetic Screen in *Drosophila* for Metastatic Behavior. *Science*. 2003 302:1227-1231

Drosophila in the lab

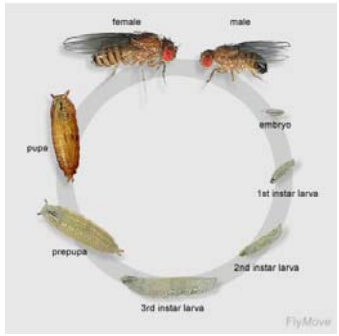
- Rearing
- Development
- Sex determination
- Mutants and repositories

Rearing

- *Drosophila* is found in all warm countries in abundance of overripe soft fruits like grapes, bananas and plums. Adult flies as well as larvae feed on the fruit juices and the yeast growing on rotting fruit.
- Since yeast represents an important part of their diet, in the laboratory *Drosophila* may be raised on many different kinds of fermenting media.

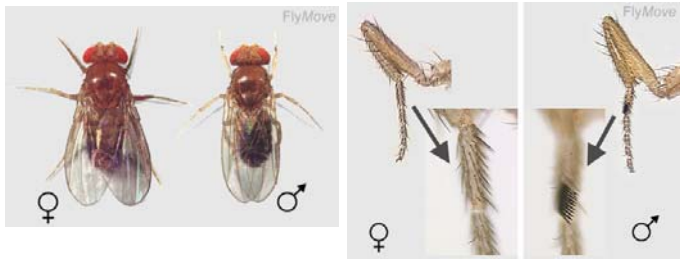
http://flyserver.uni-muenster.de/

Life cycle



- At room temperature – *i.e.* 25°C – generation time is about 10 days:
  1 day embryogenesis
  1 day first instar larvae
  1 day second instar larvae
  2-3 days third instar larvae
  5 days pupal stage

Adults



Males are usually smaller than females
Sexual characters: genitalia and combs on prothoracic legs

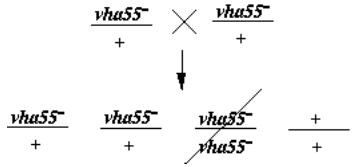
Balancer chromosomes

- To prevent divergence of the character and the marker mutation, geneticians have established flies with scrambled chromosomes which will not recombine with regular chromosomes; these chromosomes are called **balancers.**
- Ideally, balancer chromosomes in the fruit fly contain the following:
  1. an inversion or inversions to suppress the recovery of viable recombination products over the length of the chromosome;
  2. a dominant phenotype that enables the inheritance of the chromosome to be tracked easily in subsequent crosses; and
  3. a recessive lethal mutation that eliminates the homozygous balancer from the population of breeding flies.

Example on how to keep a mutation

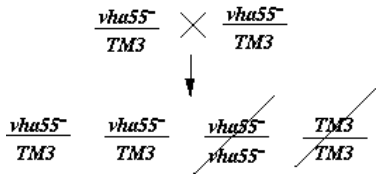
- We have found a mutant deficient for calmoduline *Cam*^{7}.
- This mutation is located on chromosome 2 and is lethal when homozygote
- If the heterozygote state is less performant than a wild homozygote, the mutation will disappear in the following generations
- Using a balancer on chromosome 2 will maintain the mutation in heterozygote flies. If we cross our *Cam*^{7} strain with a *CyO* strain (balancer on chromosome 2). If we cross *Cam*^{7} with CyO, only heterozygote individuals will survive. And the balancer chromosome CyO will not recombine so that the mutation remain stable.

What's a balancer chromosome?



- Any lethal recessive gene is likely to be lost from a population, because it confers a disadvantage on the progeny of heterozygotes. Imagine that we discover a lethal allele of our gene, vha55-. How can we keep it alive, perhaps for years? Here's what happens when we try to breed two heterozygotes

What's a balancer chromosome? (2)

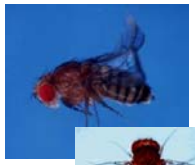


- If you cross it with a balancer chromosome, the only class of progeny which survives is identical to the parents. So once we've gone to the initial effort of crossing-in our balancer chromosome (in this case, TM3), the mutation can be kept for many years with minimal effort.
- In addition, the balancer chromosome is scrambled (to prevent recombinations) and bears a dominant mutation marker.

Marker mutations

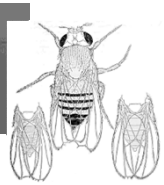

white

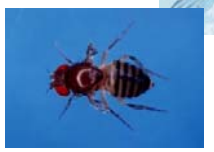

bar


curly

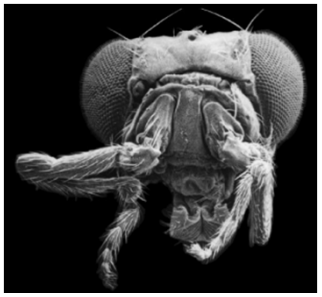

bithorax


wingless


notch


vestigial

Banks of mutants



Antennapedia mutant

- Bloomington (Indiana): 7700 mutants
- Hungary: P elements
- Ohio/Arizona: *Drosophila* species
- Japan, etc…

P elements

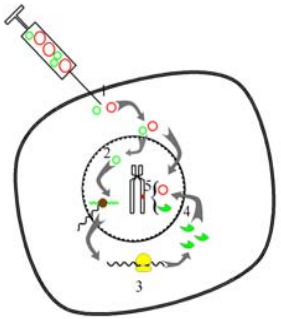
- P elements = transposons
- Used for mutagenesis
- Used to mark and induce gene mutations
  - P[*lacZ*]
  - P[*GAL4*]

Barbara McClintock

P elements

- Definition:
  - DNA transposons that « jump » from one place to the other (« cut and paste » mechanism)
  - It codes for a transposase that excises a sequence flanked by a specific DNA motif
  - This sequence is randomly inserted elsewhere in the genome
- P elements have first appeared in *Drosophila* in the middle of the twentieth century. Within 50 years, they have spread through every population of the species. Artificial P elements can be used to insert genes into Drosophila by injecting the embryo.
- *Drosophila* has other transposons: piggyBac, Hobo, etc.

Modified P elements



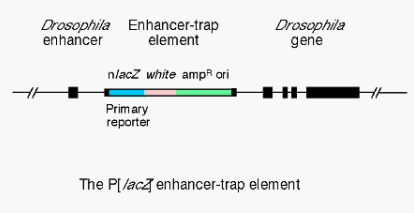
1. Inject embryo with modified P elements and RNA for transposase
2. Transcription of the helper
3. ARNm -> transposase
4. Insert transposon
5. Replication

Use of modified P elements

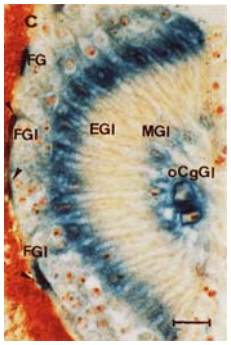
- Historically, P elements were used to induce a mutation and insert a reporter gene
- The content of P[*X*] will be expressed if it is inserted:
  - into an expressed gene; most of the time, the gene will be inactivated = mutagenesis.
  - Near a regulatory sequence. It will tag the (unknown) gene.
- Reporter genes are chosen so that their expression is easy to track. A P element and a reporter gene are called « **enhancer trap** ».

Enhancer trap P[*lacZ*]

- Reporter gene is a nuclear ß-galactosidase
- The presence of the enzyme in a cell is revealed by histochemistry (blue coloration).



The P[*lacZ*] enhancer-trap element

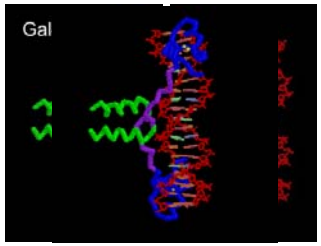


Drosophila: reporter gene expression in glial cell types of the lamina (source: FLYBRAIN)

Enhancer trap P[*Gal4*]

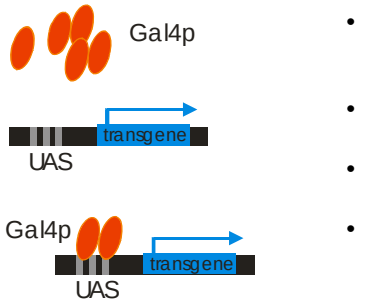
- Based on a signalisation system found in yeast
- Use P elements for transgenesis
- Inactive *per se* (GAL4 does not noticeably impact on the physiology of the cells expressing it).
- Active only if we cross two *Drosophila* strains:
  - Strain 1: reporter gene producting Gal4
  - Strain 2: UAS sequence driving gene « X »
- Advantage: flexible combinations

Gal4 - UAS



- The yeast GAL4 Protein is a transcriptional activator that binds to DNA.
- DNA shown using Sticks with the bases colored Shapely, and the backbone colored red.
- The two subunits of the symmetric dimer are shown using the Backbone display. The DNA recognition module is blue; the linker region is purple; and the dimerization element is green. The two metal ions bound to each subunit are shown as yellow balls.

Yeast: Gal4 -> UAS



- Gal4 protein regulates the the expression of genes with a USA promoter
- Gal4 / UAS is not present in insects
- Gal4 can accumulate in a cell without damaging it.
- The gene behind UAS is not expressed in the absence of Gal4

UAS = upstream regulated activating sequence

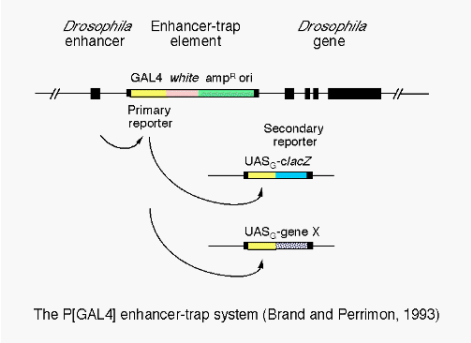
GAL4 = transcription activator

http://info.bio.cmu.edu/Courses/BiochemMols/MolBiol/1D66.html

P[UAS X]

- P[Gal4] need a partner to "convert" Gal4 into the product of another reporter gene.
- P[UAS X] : Example P [UAS GFP]
- P[UAS TNT]
- P[UAS *Reaper*]
- P[UAS *tra*]
- …

P[*GAL4*] x P[UAS X]



The P[GAL4] enhancer-trap system (Brand and Perrimon, 1993)

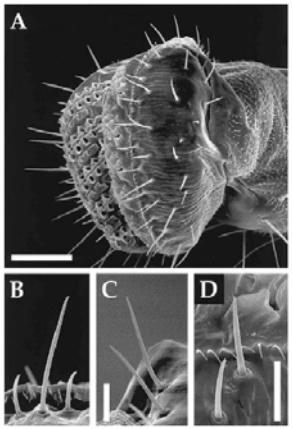
Limitation of P[*GAL4*] x P[UAS X]

- Imperfect to study an expression profile because PGAL4 does not characterize strictly the gene affected or trapped. These strains can be used also to induce:
  - Mutations by imprecise excision
  - To express a transgene into a given tissue
- P[Promoter Gal4] x P[UAS X]
- P[native gene with Gal4 inserted]

P[Promoter Gal4]

- Used to study the expression profile of a gene identified by its sequence.
- More precise … it reflects the gene expression because we use the promoter sequence of that gene.

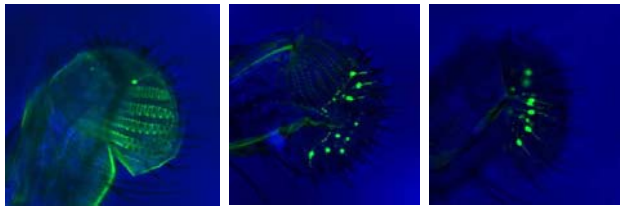
Application to taste neurones



- Taste sensilla on the proboscis
- 3 types: L (long), S (small), I (intermediate)
- 4 taste neurones / sensillum + 1 mechanoreceptor
- Exception: type i = 2 neurones

From Makoto Hiroi, Zool J 2003

Grs expressed in the proboscis

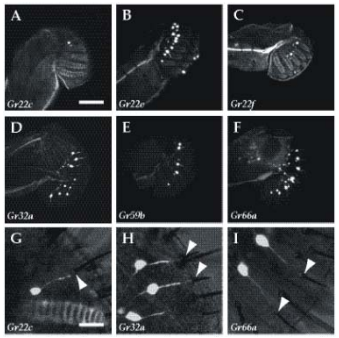


Gr22c *Gr32d1* *Gr66c1*

- Construction : putative promoter of Grxx-Gal4] x P[UAS-eGFP]

From Makoto Hiroi, Zool J 2003

Expression of Grs / UAS-Gal4



- The UAS-Gal4 is more sensitive than the detection of RNAs (PCR, hybridation *in situ* : impossible to visualize)
- It does not allow us to dose the expression level of the transcripts nor to know when they have been transduced

From Makoto Hiroi, Zool J 2003

Modified GFP

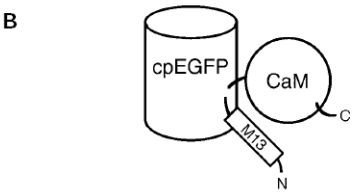
Cell, Vol. 112, 271–282, January 24, 2003

Two-Photon Calcium Imaging Reveals an Odor-Evoked Map of Activity in the Fly Brain

Jing W. Wang, Allan M. Wong, Jorge Flores, Leslie B. Vosshall, and Richard Axel

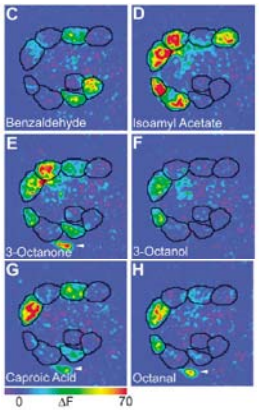
Summary
An understanding of the logic of odor perception requires a functional analysis of odor-evoked patterns activity in neural assemblies in the brain. We have developed a sensitive imaging system in the *Drosophila* brain that couples two-photon microscopy with the specific expression of the calcium-sensitive fluorescent protein, G-CaMP. At natural odor concentration, each odor elicits a distinct and sparse spatial pattern of activity in the antennal lobe that is conserved in different flies. Patterns of glomerular activity are similar upon imaging of sensory and projection neurons, suggesting the faithful transmission of sensory input to higher brain centers. Finally, we demonstrate that the response pattern of a given glomerulus is a function of the specificity of a single odorant receptor. The development of this imaging system affords an opportunity to monitor activity in defined neurons throughout the fly brain with high sensitivity and excellent spatial resolution.

G-CaMP = modified GFP

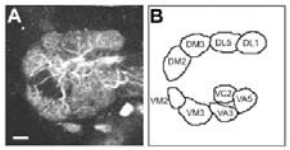


Nakai et al, 2001

G-CaMP and olfactory glomeruli



- GH146-Gal4 induces the expression of G-CaMP in primary olfactory neurons and in the projection neurons of the antennal lobe.
- Activation of the glomeruli by an odorant induce a surge of calcium and a change of fluorescence.



Compare signal in/out in the AL

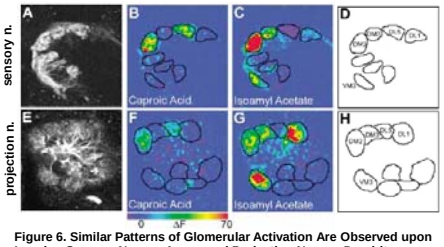


Figure 6. Similar Patterns of Glomerular Activation Are Observed upon Imaging Sensory Neuron Axons and Projection Neuron Dendrites
(**A–D**) Fly bearing the *Or83b-Gal4* and the *UAS-GCaMP* transgenes. **(A)** An image obtained prior to odor stimulation reveals the glomerular structure. The DL1 glomerulus is weakly labeled by *Or83b-Gal4*. Glomerular activity in response to caproic acid **(B)** and isoamyl acetate **(C)**. Odor concentrations were 33% SV. **(D)** A schematic of identified glomeruli in this optical plane. **(E–H)** Fly bearing the *GH146-Gal4* and the *UAS-GCaMP* transgenes. **(E)** The glomerular structure shown in grayscale. Glomerular activity in response to caproic acid **(F)** and isoamyl acetate **(G)**. Odor concentrations were 33% SV. **(H)** A schematic of identified glomeruli in this optical plane.

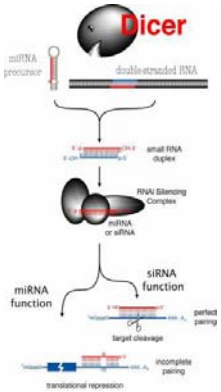
(I) Concordance of glomerular responses in flies expressing G-CaMP in sensory neurons and projection neurons. Nine odorants at 36% SV were applied to preparations of flies bearing the *Or83b-Gal4* (green rectangles) and *UAS-GCaMP* transgenes. Each glomerulus that showed at least 20% F/F in G-CaMP labeled PNs (blue rectangles) in response to less than 20% SV for each odor was also activated in flies in which sensory neurons were labeled with G-CaMP. The VM1 and DP1m glomeruli are not labeled by *Or83b-Gal4*.

Inhibit the expression of genes

- Gene disruption
- Pharmacological inhibition
- Microinjection of antibodies
- Dominant mutations

- … and RNAi = selective « inactivation » of a gene

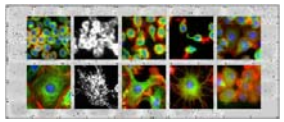
RNAi



- A cellular pathway mitigates post-transcriptional gene silencing. Long double-stranded RNA or microRNA hairpin RNAs are processed by the cellular factor Dicer to form siRNAs or miRNAs. These RNAs are used by a RNAi silencing complex to degrade mRNA (RNAi) or to translationally repress the mRNA (siRNA). Little is known about the mechanisms behind small RNA directed silencing, and even less about the factors which participate in these two processes.

http://web.mit.edu/mmcmanus/www/RNAi.html

RNAi screen



- **Systematic RNAi screens in *Drosophila* cells identify known and novel gene functions required for specific cell-biological processes.**

  Foreground: Systematic RNAi screens in cultured Drosophila cells identified gene functions associated with distinct morphological phenotypes. Automated fluorescence microscopy was used to detect organization of filamentous-actin (red), microtubules (alpha-tubulin, green) and DNA (blue), allowing unprecedented genetic dissection of cellular morphogenesis. Specific defects included those in cell adhesion, lamellipodia formation, cell cycle progression and cytokinesis.

- **Kiger, A. A., B. Baum, S. Jones, M. Jones, A. Coulson, C. Echeverri, N. Perrimon.** 2003. A functional genomic analysis of cell morphology using RNA-interference. *J. Biol.*, 2: 27.

Inducible expression systems

- Hsp – heat shock proteins
- GeneSwitch – modified GAL4 + RU486
- Gal80 – inhibition Gal 4 / temperature
- Mosaic – expression / cellular lines

Heat shock proteins

- A thermal stress induces the synthesis of proteins (chaperones)
- The promoter of one of these proteins has been introduced in a P element to induce the expression of a reporter gene

Ex: hsp70-Gal4
thermal shock → Gal4 in all cells

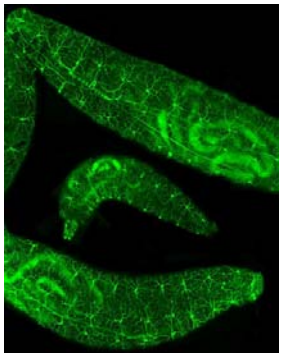
PNAS | September 1, 1984 | vol. 81 | no. 17 | **5509-5513**

Inducible Transcription and Puffing in Drosophila melanogaster Transformed with hsp70--Phage Hybrid Heat Shock Genes

Robert S. Cohen and Matthew Meselson

A series of hsp70-phage lambda hybrid genes having various amounts of 5' flanking DNA was introduced into the germ line of Drosophila melanogaster by P-element-mediated transformation. Heat-induced transcription was normal in lines transformed with hsp70- genes having 194 and 146 base pairs of DNA upstream from the mRNA initiation site.

P[promoter-heat shock-*GFP*]



- The image shows a confocal montage of *Drosophila* larvae expressing EGFP under the control of a 1kb genomic region 5' of the *pickpocket* gene. Labeling is observed in a segmentally repeated subset of dendritic arborization (da) sensory neurons. The dendrites of these neurons provide a complete and non-redundant tiling of the epidermis (axons can be seen projecting to the CNS). Ablation experiments suggest that homotypic dendrites repel each other where they meet.

Grueber WB, Ye B, Moore AM, Jan LY, Jan YN. 2003. Dendrites of distinct classes of *Drosophila* sensory neurons show different capacities for homotypic repulsion. *Curr Biol* 13:618-626

A schematic diagram of the *ppk enhancer* construct. Red indicates the enhancer region, black indicates the minimal heat shock promoter, and green indicates the EGFP cDNA.

GeneSwitch/UAS

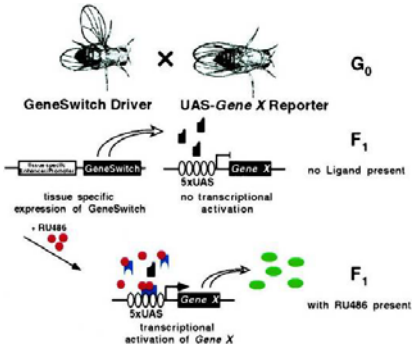
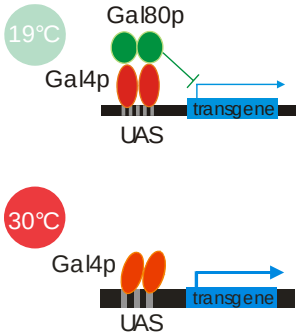


Figure 1. The GeneSwitch/UAS expression system in *Drosophila*. Driver lines expressing the transcriptional activator GeneSwitch in a tissue-specific fashion are crossed to UAS-reporter lines with genomic inserts of a target gene fused to five GAL4-binding sites arrayed in tandem (5× UAS). In the absence of an activator, the GeneSwitch protein is expressed in target tissues but remains transcriptionally silent (black); *Gene X* is therefore not expressed. However, after systemic application of RU486 (red), the GeneSwitch protein becomes transcriptionally active (blue), mediating expression of *gene X* (green) in only those tissues expressing GeneSwitch.

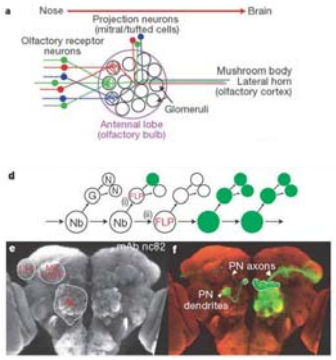
Proc. Natl. Acad. Sci. USA. 2001 October; 98 (22): 12596 - 12601

Regulation of Gal4: Gal80



- Gal80 is a protein that inhibit the induction of UAS by Gal4.
- Gal80 structure is modified at 30°C and fails to repress Gal4.
- Thus, we can express a transgene in a specific tissue AND control its expression (like GeneSwitch)

Mosaïc – MARCM



- The principle of mosaic techniques is to genetically mark a mother cell during development.
- All cells issued from the division of this cell will be labeled.
- MARCM uses a flippase (for ex under the control of hsp) which inverts a DNA sequence

see:
BLAIR S. S. 2003. Genetic mosaic techniques for studying Drosophila development. *Development* 130: 5065-72.
See also:
Wong et al. 2002. Spatial representation of the glomerular map in the Drosophila protocerebrum. *Cell* 109: 229-41.
and
Marin et al.. 2002. Representation of the glomerular olfactory map in the Drosophila brain. *Cell* 109: 243-55.

Physiology of the olfactory receptors

- Before 2000, data on olfaction of Drosophila are scarce (anosmic mutants, EAG)
- In2000, two teams publish simultaneously a list of putative olfactory receptors in Drosophila, obtained thanks to genomic approaches (structure of the DNA and prediction of the structure of the proteins.
- This list is extended to 60 putative receptors (Ors).
- We needed a physiological test.
- Stortkül et al. overexpress one of these receptors in all sensilla of the antenna and show that EAG responses and the behavior are modified in response to one odor.
- De Bruyne et al develop a technique to record from single sensilla which they use first on the maxillary palps and then on the antenna.

Physiology of olfactory sensilla

Neuron, Vol. 30, 537–552, May, 2001, Copyright ©2001 by Cell Press

Odor Coding in the *Drosophila* Antenna

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and John R. Carlson*
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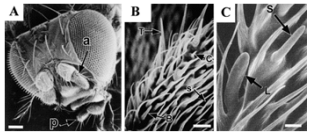
Yale University
New Haven, Connecticut 06520

Summary

Odor coding in the *Drosophila* antenna is examined by a functional analysis of individual olfactory receptor neurons (ORNs) in vivo. Sixteen distinct classes of ORNs, each with a unique response spectrum to a panel of 47 diverse odors, are identified by extracellular recordings. ORNs exhibit multiple modes of response dynamics: an individual neuron can show either excitatory or inhibitory responses, and can exhibit different modes of termination kinetics, when stimulated with different odors. The 16 ORN classes are combined in stereotyped configurations within seven functional types of basiconic sensilla. One sensillum type contains four ORNs and the others contain two neurons, combined according to a strict pairing rule. We provide a functional map of ORNs, showing that each ORN class is restricted to a particular spatial domain on the antennal surface.

Antennal olfactory system (1)

- The antenna is densely packed with olfactory sensilla (5000?)
- Each sensilla house 2 to 4 olfactory receptor neurones (ORNs)
- These sensilla belong to different morphological types: basiconica, trichoids, coeloconica



De Bruyne et al (2001) Neuron 30: 537-552

Figure 1. Olfactory Sensilla
(A) Olfactory sensilla on the third segment of the antenna (a) and the maxillary palp (p). Scale bar, 100 mm. (B) Third antennal segment: basiconic ("B"), trichoid ("T"), and coeloconic ("C"). Scale bar, 5 mm. (C) The two varieties of basiconic sensilla: large (L) and small (S). Scale bar, 2 mm.

Record the response of ORNs

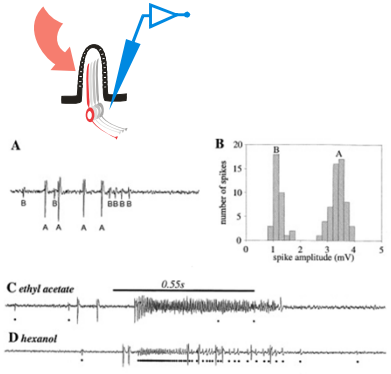


Figure 2. Extracellular recordings from basiconic sensilla
(**A**) Spontaneous activity (1 s) of a basiconic sensillum later classified as ab3. Individual action potentials (spikes) are labeled A or B according to their amplitude.
(**B**) The bimodal distribution of spike amplitudes, as measured from peak to trough, in the recording shown in (A). Data shown represent 90 spikes from 6 s of the recording. "A" and "B" indicate subpopulations of spikes attributed to neurons A and B.
(**C and D**) Two 1500 ms traces of recordings from one sensillum, later classified as ab2, demonstrating different responses of the two neurons to different odors. Large action potentials, from the A neuron, increase in frequency in response to ethyl acetate (C). Dots indicate smaller action potentials from the B neuron, which is not excited by ethyl acetate but which responds to hexanol (D).
For odor stimulation (0.55 s, horizontal bar), air was expelled from a syringe over filter paper containing 20 µl of odorant, diluted 10-2 in paraffin oil.

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ORNs response profiles

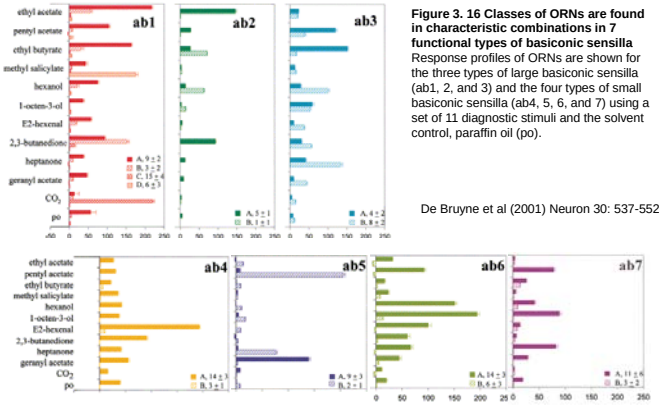
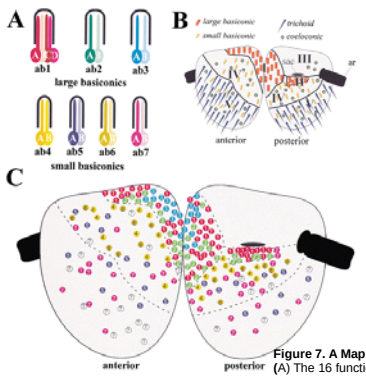


Figure 3. 16 Classes of ORNs are found in characteristic combinations in 7 functional types of basiconic sensilla
Response profiles of ORNs are shown for the three types of large basiconic sensilla (ab1, 2, and 3) and the four types of small basiconic sensilla (ab4, 5, 6, and 7) using a set of 11 diagnostic stimuli and the solvent control, paraffin oil (po).

De Bruyne et al (2001) Neuron 30: 537-552

Functional cartography



- The responses obtained with a restricted odor panel allow to separate sensilla basiconica into 7 classes
- Each class is characterized by a unique response profile
- Systematic recordings allow to establish a functional map

Figure 7. A Map of Neuronal Classes on the Antennal Surface
(**A**) The 16 functional classes of ORNs, 7 functional types of sensilla. (B) Regions of the antenna. (C) Distribution of functional types of sensilla. Each circle represents a recording from one sensillum.

De Bruyne et al (2001) Neuron 30: 537-552

Results

- Establish response profiles of ORNs
- Comparable results obtained on the maxillary palp
- This is the first quantitative and qualitative description of the physiological responses of ORNs in *Drosophila*. The responses are similar qualitatively to what has been observed on larger insects.

- But:
  - Response of ORNs in other sensilla ?
  - Where does specificity come from? Ors? OBPs?
  - Is there a connection between the response profile of the ORNs and that of the Ors (60 Ors)?

The « empty neuron » approach…

Cell, Vol. 117, 965–979, June 25, 2004, Copyright ©2004 by Cell Press

The Molecular Basis of Odor Coding in the *Drosophila* Antenna

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Summary

We have undertaken a functional analysis of the odorant receptor repertoire in the *Drosophila* antenna. Each receptor was expressed in a mutant olfactory receptor neuron (ORN) used as a "decoder," and the odor response spectrum conferred by the receptor was determined in vivo by electrophysiological recordings. The spectra of these receptors were then matched to those of defined ORNs to establish a receptor-to-neuron map. In addition to the odor response spectrum, the receptors dictate the signaling mode, i.e., excitation or inhibition, and the response dynamics of the neuron. An individual receptor can mediate both excitatory and inhibitory responses to different odorants in the same cell, suggesting a model of odorant receptor transduction. Receptors vary widely in their breadth of tuning, and odorants vary widely in the number of receptors they activate. Together, these properties provide a molecular basis for odor coding by the receptor repertoire of an olfactory organ.

Pattern of expression of Ors

- Each olfactory neurone would express only one Or
- More than 60% of the neurons express in addition Or83b which is a co-partner
- Creating Gal4 lines allowed Carlson's group to identify receptors expressed within the functional types established by de Bruyne *et al.*

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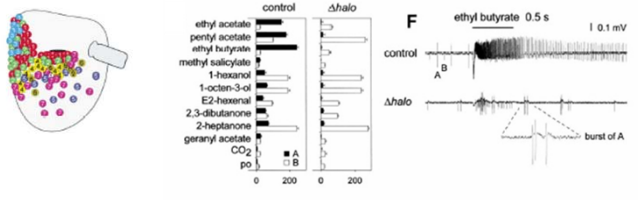
Δ*halo* mutant



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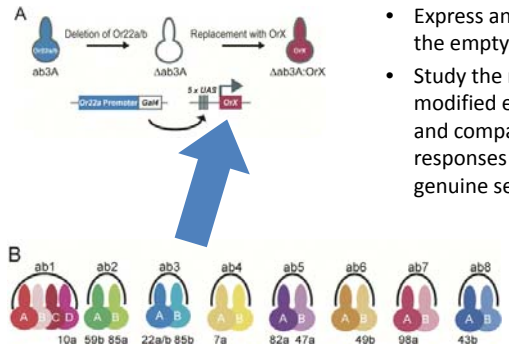
- Δ*halo* mutation affects the expression of Or22a/b
- These receptors are expressed in ORNs into sensilla ab3
- The corresponding ORN does not respond to odors anymore.

Ors substitution



- Express another Or into the empty neuron ab3A
- Study the response of the modified empty neuron and compare it with responses observed in the genuine sensilla

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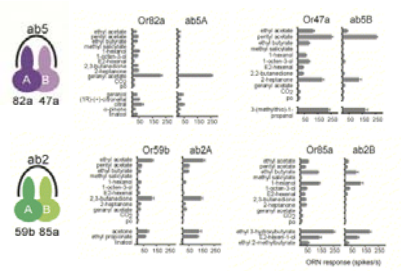
Ors empty neuron / genuine



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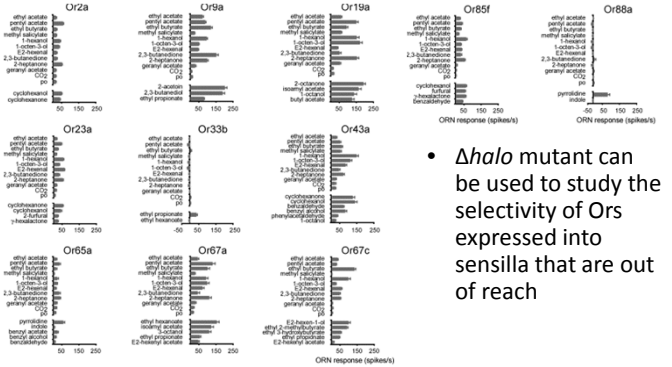
- The response profile in the modified neurone is identical to the responses obtained in the original ORNs

Ors / unknown sensilla



- Δ*halo* mutant can be used to study the selectivity of Ors expressed into sensilla that are out of reach

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Functional properties

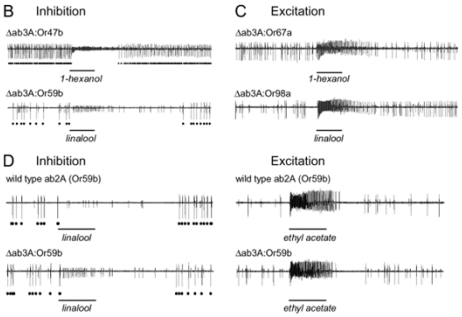

Figure 4. Spontaneous Firing Rate and Signaling Mode of ORNs Are Determined by the Odorant Receptor
(B) Inhibitory responses of ab3A:OrX neurons (large spikes; positions indicated by dots). The excitatory response of the ab3B neuron, which resides in the same sensillum, is also visible (small spikes).
(C) Excitatory responses of ab3A:OrY neurons (large spikes) stimulated with the same odorants that evoked inhibition in different, ab3A:OrX neurons in (B).

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- Or expression change the behavior of a neuron in the absence of odor and the response mode(excitation/inhibition).

Qualitative coding

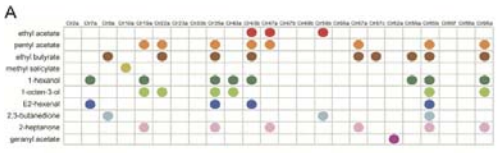

Figure 7. Odor Coding by the Antennal Odorant Receptor Repertoire
(A) Table listing receptors by cytogenetic location. Colored dots indicate strong responses (as defined by a rate of 100 spikes/s following stimulation with a 10-2 dilution).

- Each olfactory receptor neuron expresses 1 receptor (Or)
- All ORNs expressing the same Or project in the same glomerulus
- Activation of glomeruli reflect the activation of Ors.
- Each odor or odor blend is characterized by a specific activation pattern.

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Quantitative coding

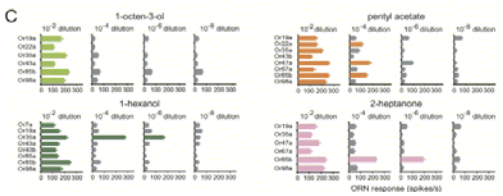

Figure 7. Odor Coding by the Antennal Odorant Receptor Repertoire
(C) Responses of receptors as a function of concentration.

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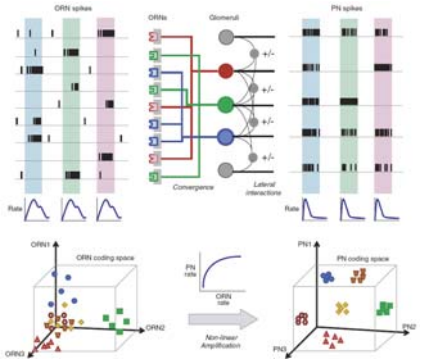
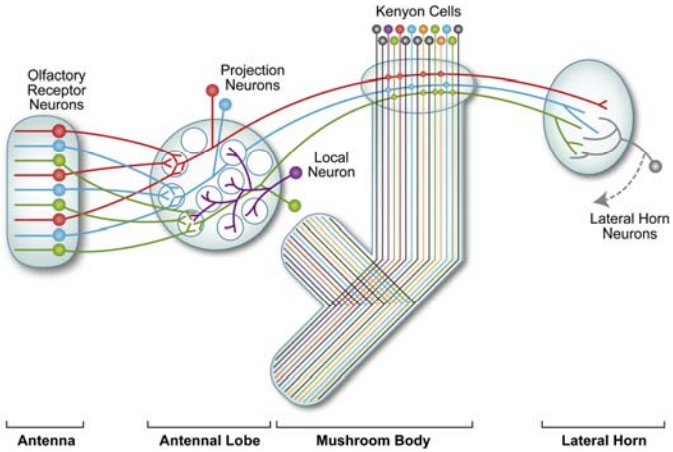
- Ors become less selective with high concentration of odors.
- Activation pattern is thus concentration -dependent.
- The functional types described here strongly depend on the choice of odors.

Olfactory computation in the AL

Olfactory receptor neurons (ORNs) produce odor-elicited spike train responses that are noisy, highly variable, and grow relatively slowly in intensity. Second-order, projection neurons (PNs) in the antennal lobe receive convergent input within glomeruli from multiple, distributed copies of the same ORN type. When driven by odor, projection neurons show responses that are more reliable and that build more rapidly than responses in the ORNs. Lateral interactions within the antennal lobe circuitry result in a non-linear amplification of ORN inputs, restructuring odor-elicited patterns across projection neurons to become more uniformly distributed and distinct from one another, and thus enable more efficient coding.



Curr Biol Raman and Stopfer 18 R29 [2008]



Cachero and Jefferis Neuron 59: 843-845 (2008)

More…?

- CO2 perception antennae: Grs (L. Vosshall)
- CO2 perception: mouthparts (K. Scott 2007)
- Expression of moth pheromone receptor (W. Leal)
- Tasting cuticular pheromone (Lacaille et al)
- Antennal lobe odor treatment (Galizia)
- Anosmic flies except but one Or and antennal lobe (L. Vosshall)
- Or topology in the membranes (L. Vosshall)
- Or choice (Carlson)
- AL & Gaba (single cell patch; Wilson and Laurent)
- Regulation of lifespan by food odors! (Libert et al., 2007)