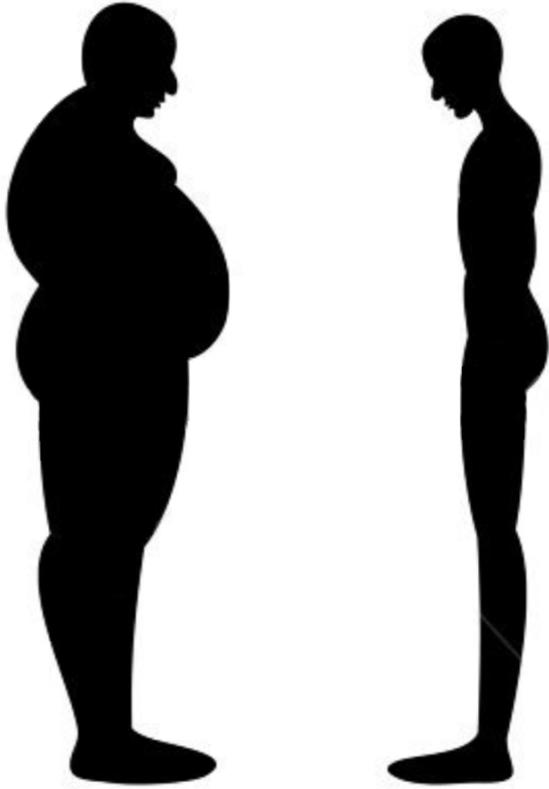


genome-wide RNAi screening and obesity



RNAi screening comes of age

Gene Silencing

RNA interference (RNAi)

CRISPR-Cas9

Gene Ontology

When was all this discovered?

1956

First Bioinformatic Database

Created after the Insulin protein sequence was made

1978

Antisense Oligonucleotides (ASOs)

Regulation of gene expression

1980

Hedgehog (Hh) Gene

Found in screen for mutations affecting Drosophila

1998

RNA Interference (RNAi)

Regulation of gene expression

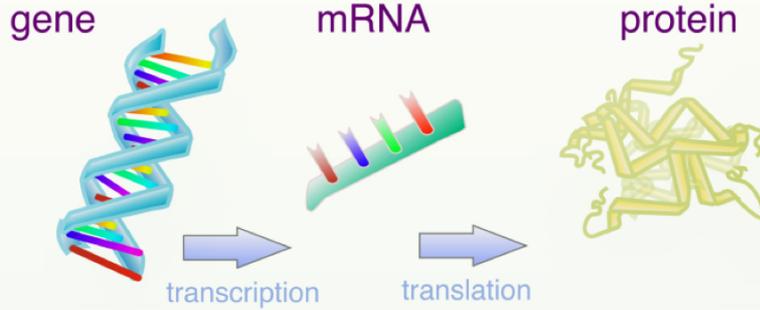
2012

CRISPR-Cas9

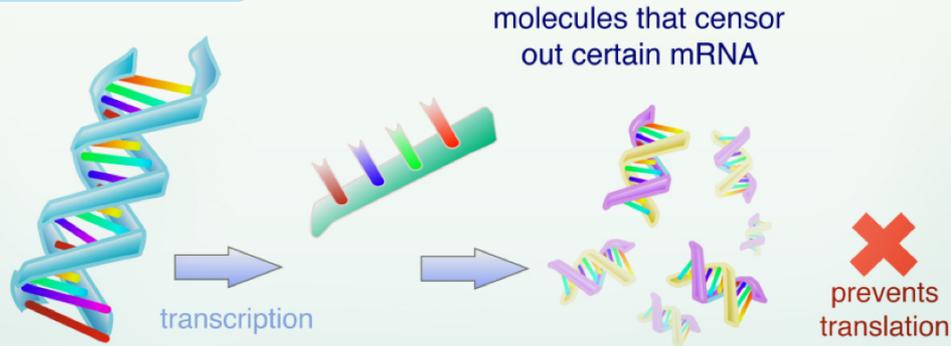
Developed as a genome editing technology

What is **gene silencing**?

normal protein production



gene silencing



Regulation of gene expression to prevent expression of a gene

What are the 2 types of **gene silencing** techniques?

Antisense Oligonucleotides (ASOs)

RNA interference (RNAi)



What are **Antisense Oligonucleotides (ASOs)**?

Synthetic nucleotides used to inhibit gene expression

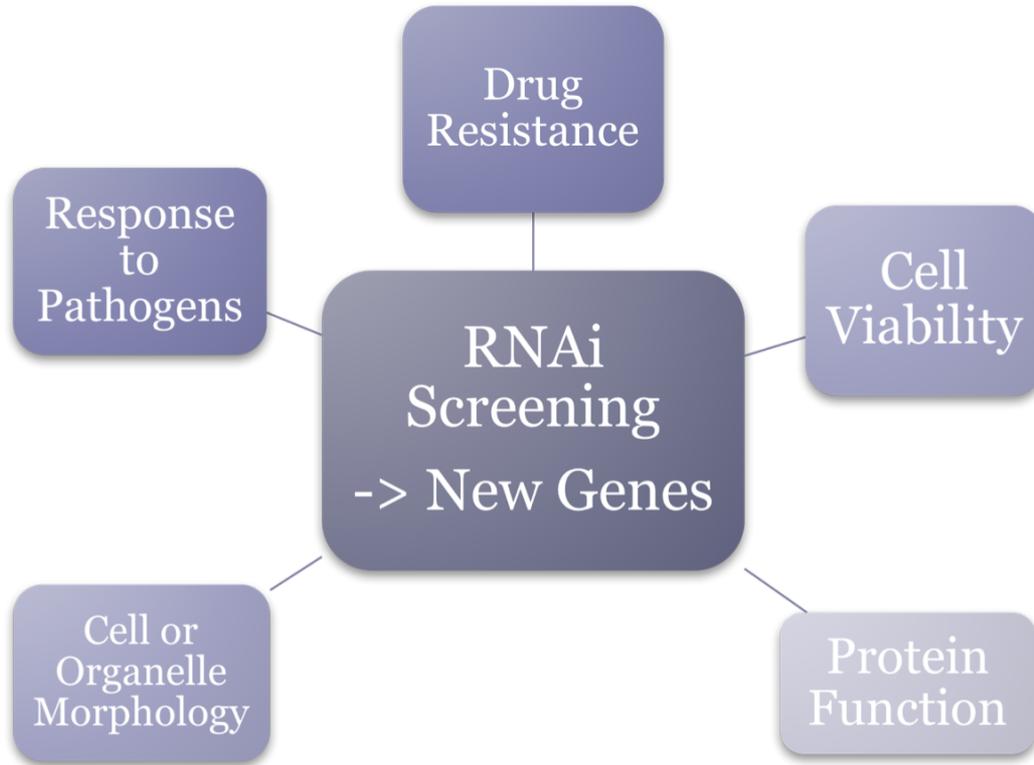
What is **RNA interference (RNAi)**?

Process in which RNA molecules inhibit gene expression

Pros & Cons of Both Techniques

	Pros	Cons
(ASOs)		
(RNAi)		

Why is **RNAi Screening** so great?



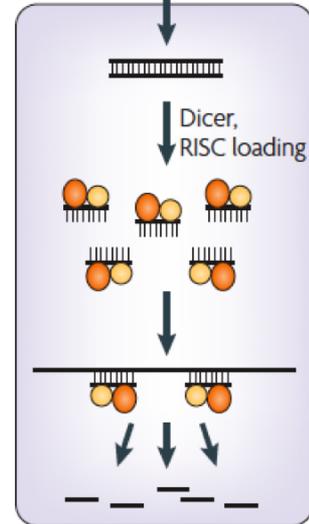
Identifies new genes involved in a variety of biological processes

Which model organism works best in RNAi Screens?

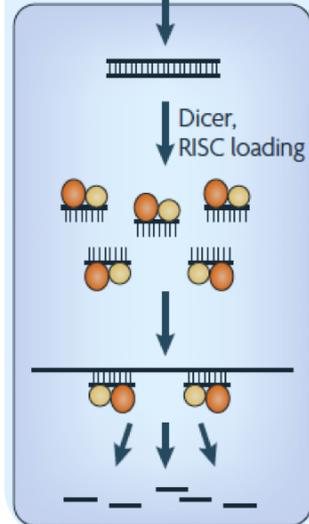
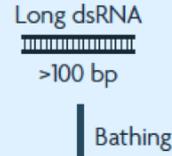
C. elegans



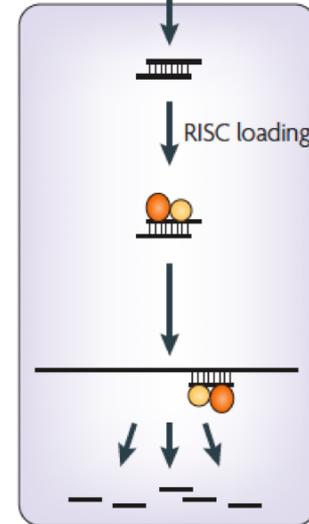
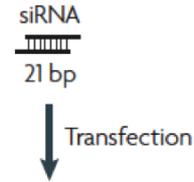
Worms



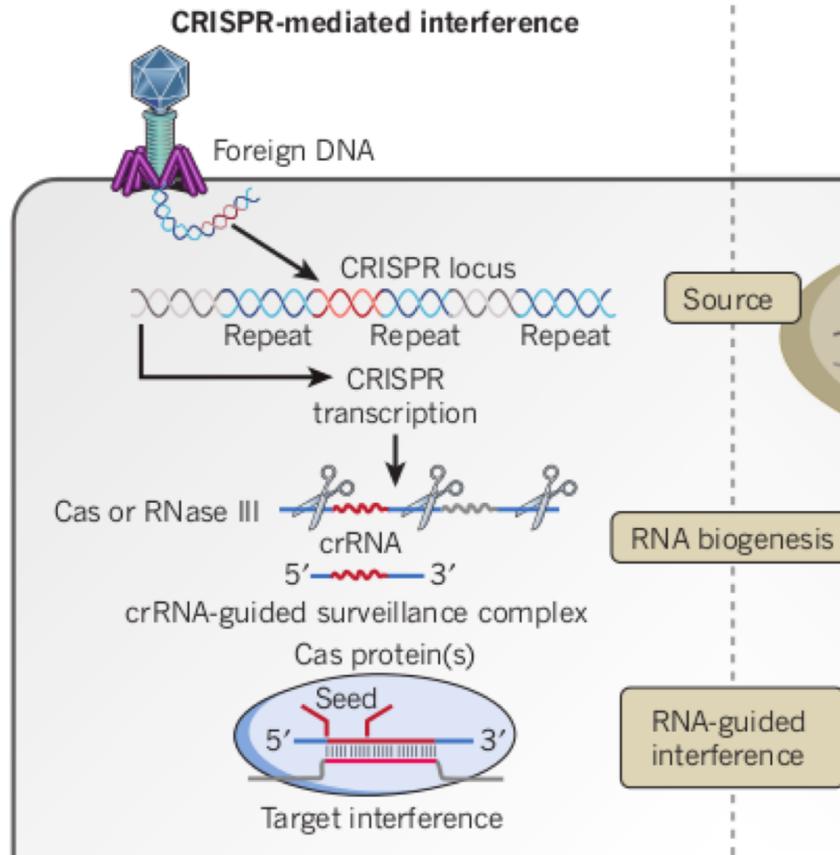
Drosophila



Humans



What is CRISPR-Cas9?

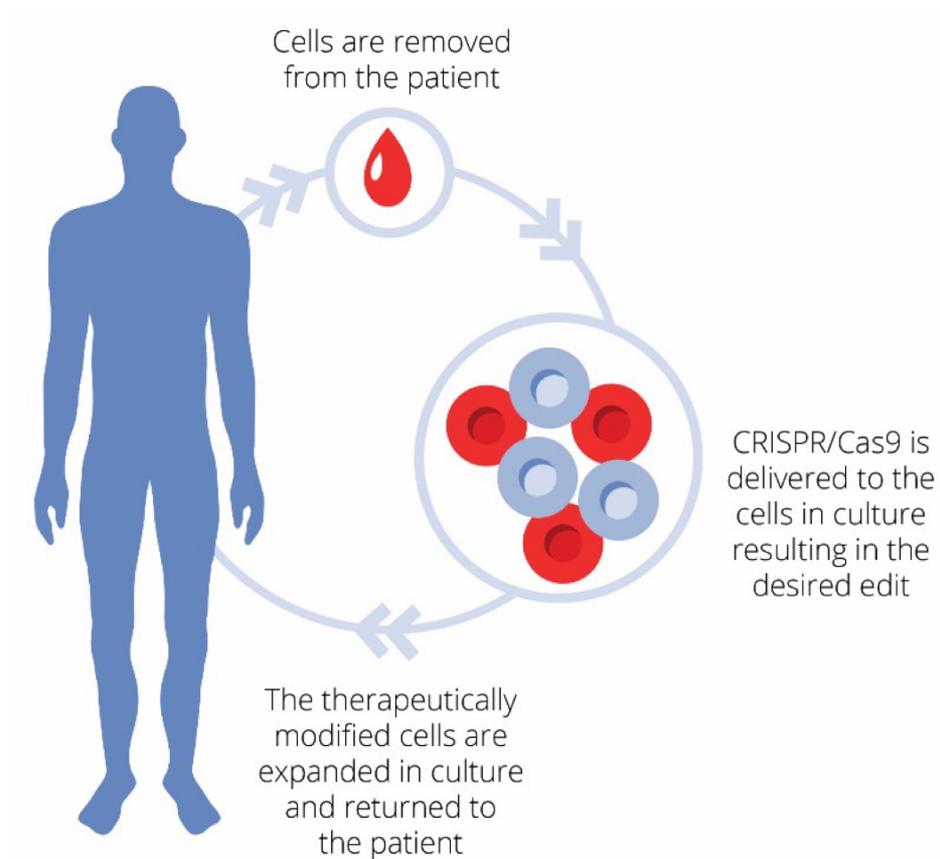


Genome editing tool that removes, adds, or alters sections of DNA sequences

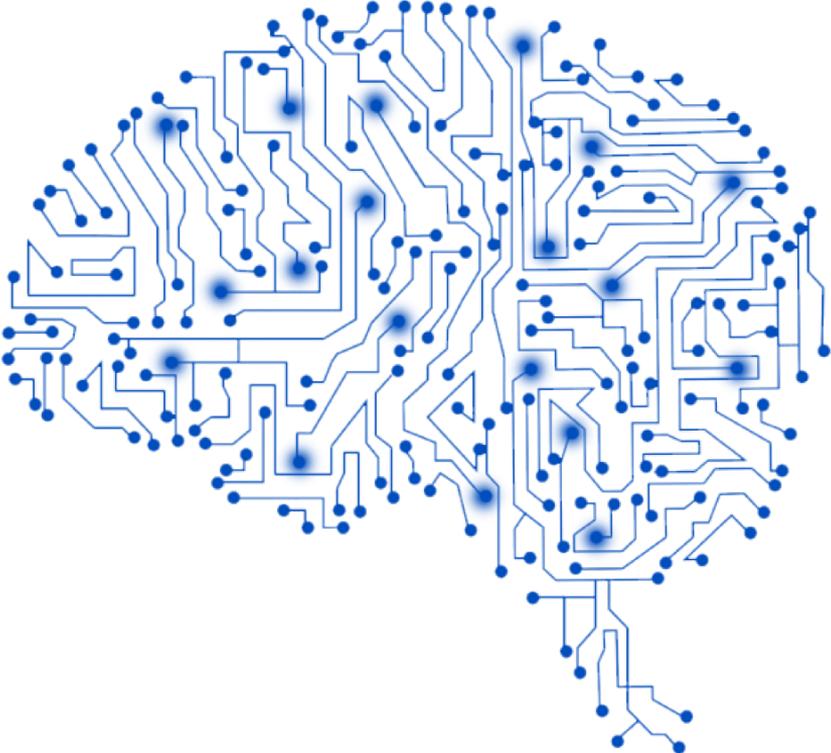
How is **CRISPR-Cas9** used to validate genome-wide screens?

By engineering knockouts in genes identified in RNAi screens

How can **CRISPR** be used to correct human mutations?



What are strategies to simplify genome-wide RNAi screen data?



Bioinformatics

What is **gene ontology**?

A gene dictionary!

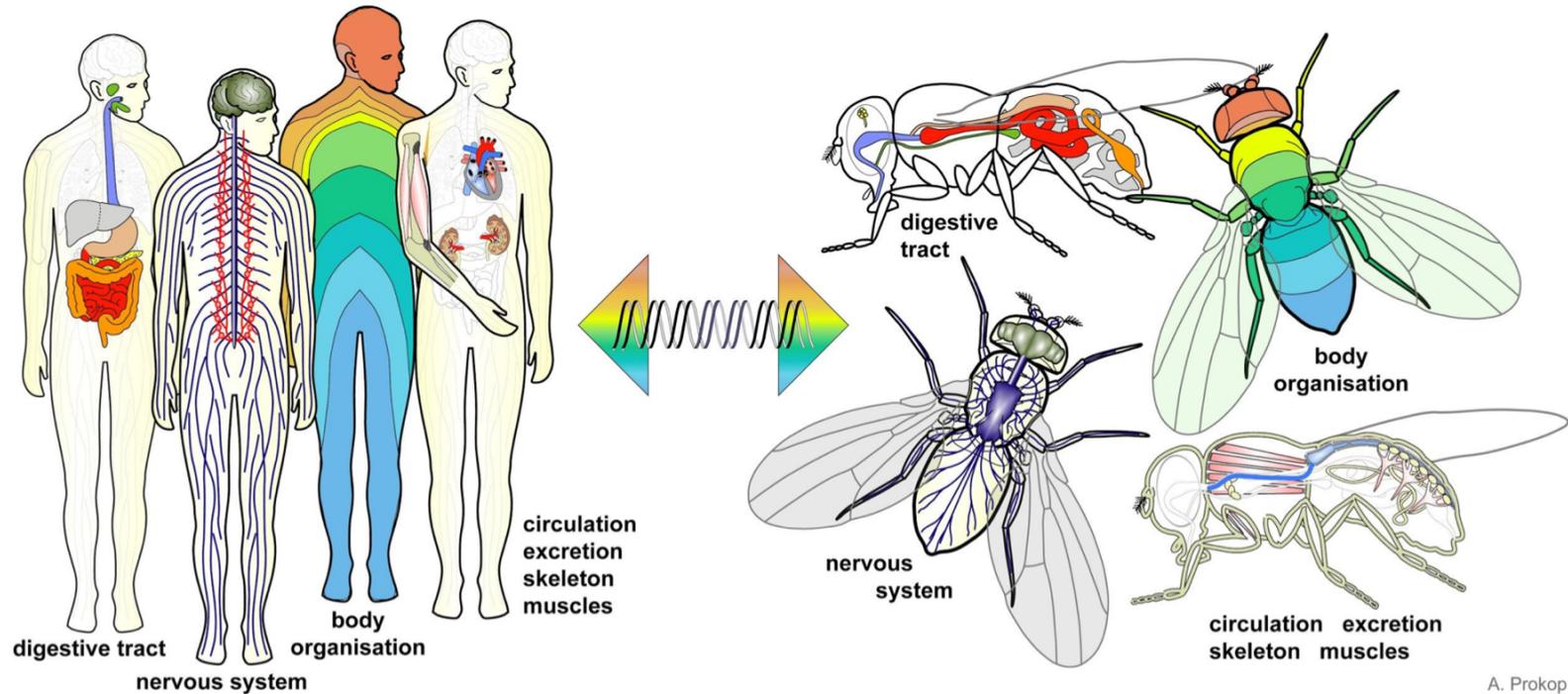
How was
gene ontology
relevant to this
study?



It was used to
find candidate
obesity genes!

Summary

Drosophila Genome-wide Obesity Screen Reveals Hedgehog as a Determinant of Brown versus White Adipose Cell Fate



A. Prokop

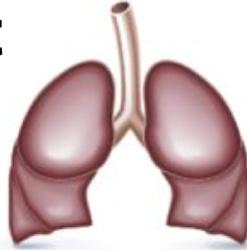
What are some diseases associated with obesity?

What are some diseases associated with obesity?

**Heart
disease**

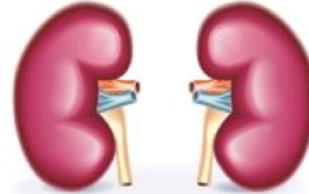


Gout



Breathing problems

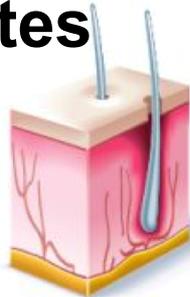
Cardiovascular



Asthma



Diabetes

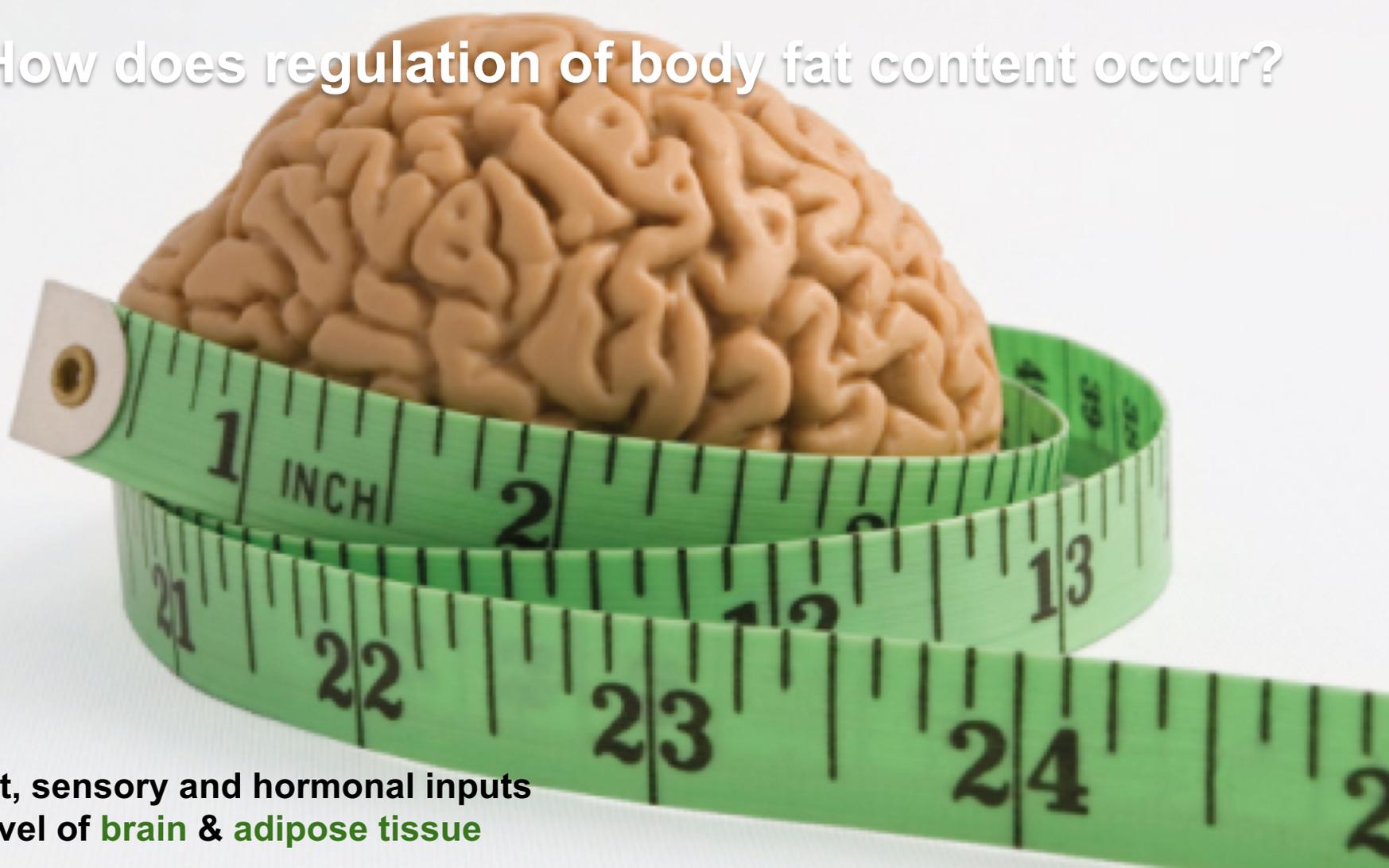


High blood pressure



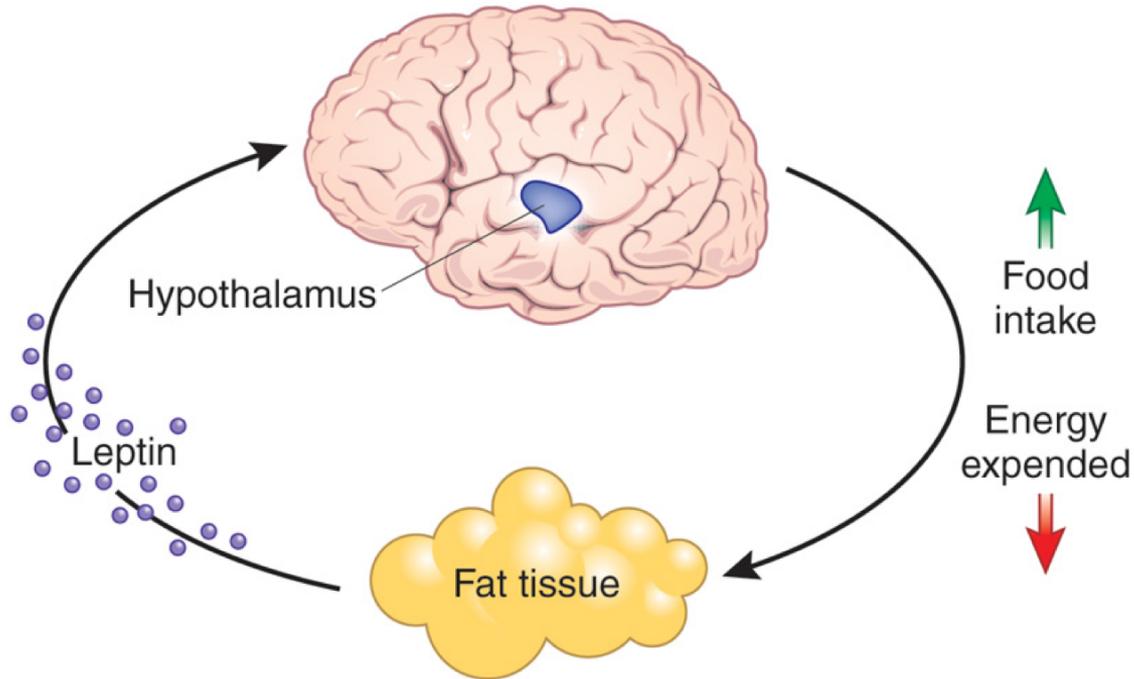
How does regulation of body fat content occur?

How does regulation of body fat content occur?



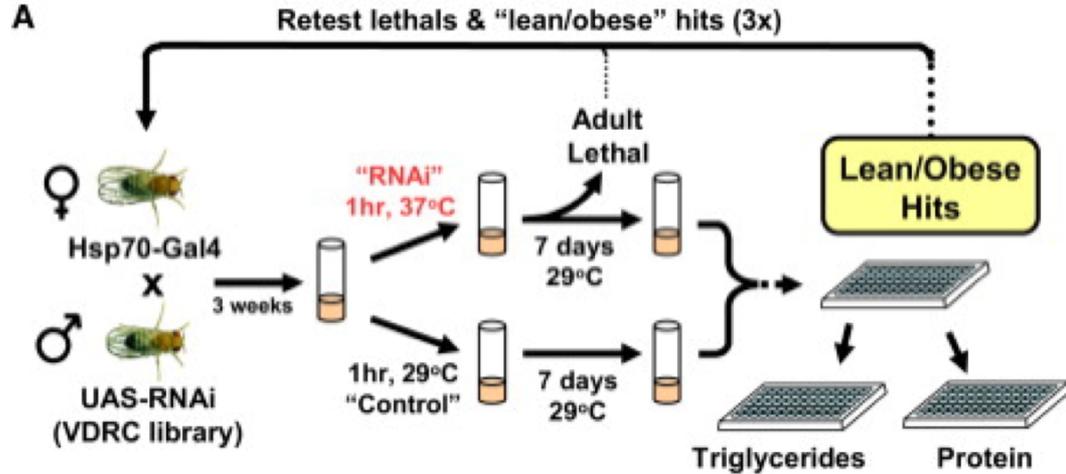
Nutrient, sensory and hormonal inputs
at level of **brain & adipose tissue**

What is leptin? (1950)



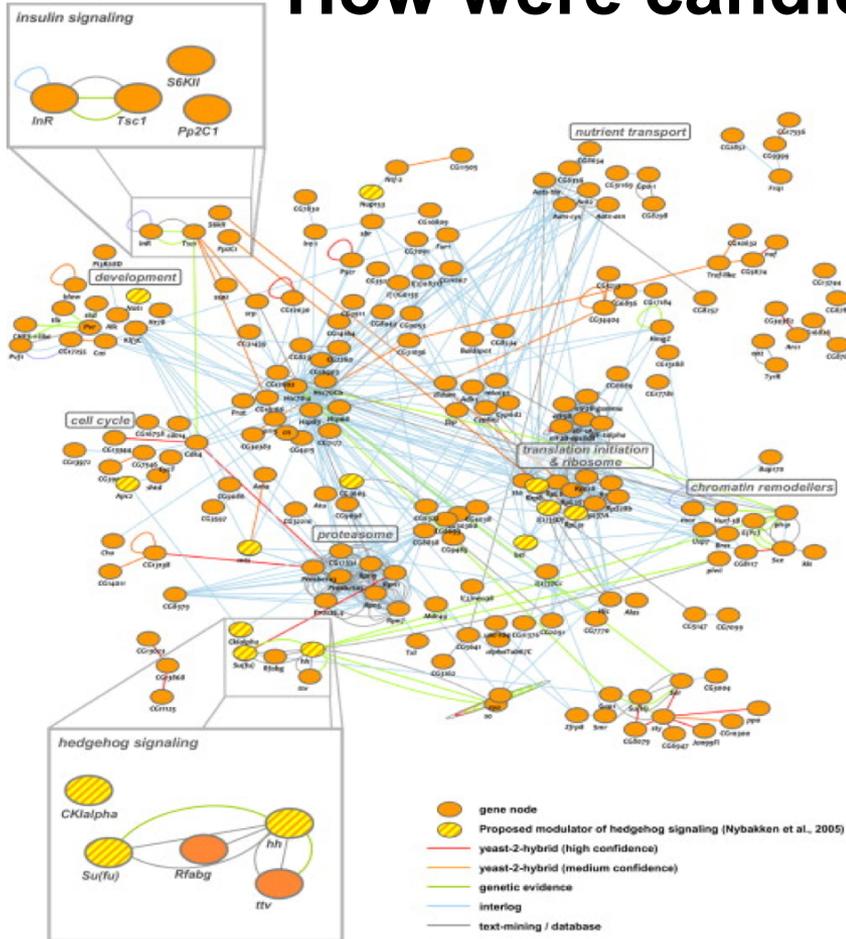
The connection between **fat** and the **brain**

What is the goal of this study?



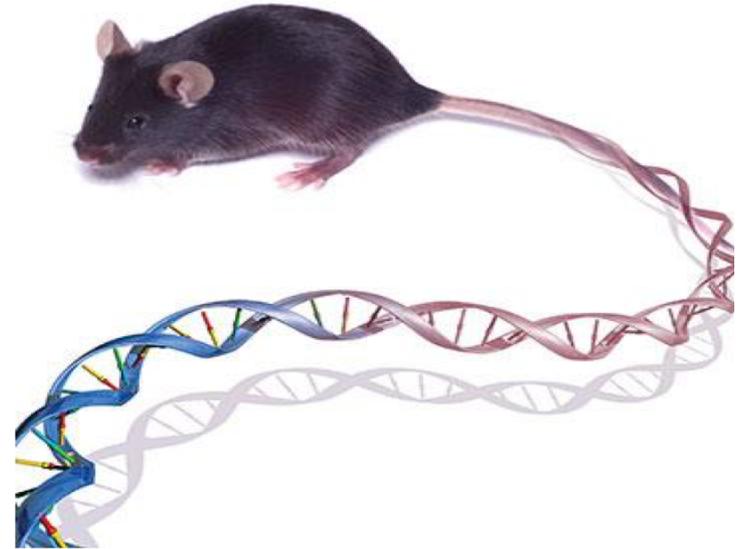
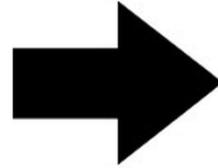
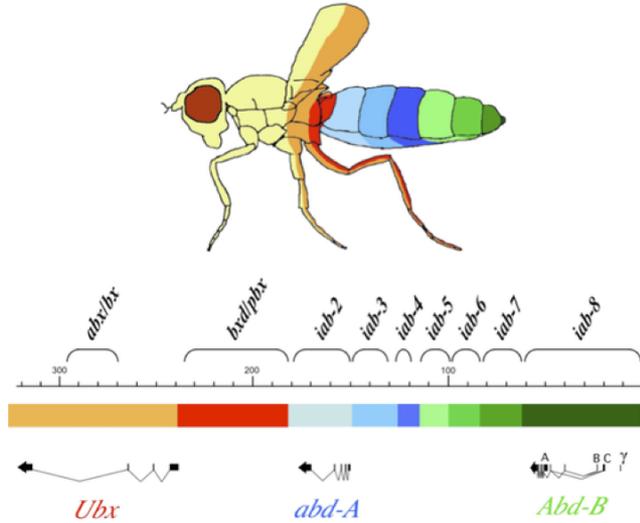
To identify **candidate obesity genes**

How were candidate genes found?



RNAi screen for fat content
in adult *Drosophila* to find
candidate genes
(516 transgenic lines)

What was the experimental set-up?



**Gene-ontology based
pathway analysis in
*Drosophila melanogaster***

**Studying “top-hit”
candidate gene in mice**

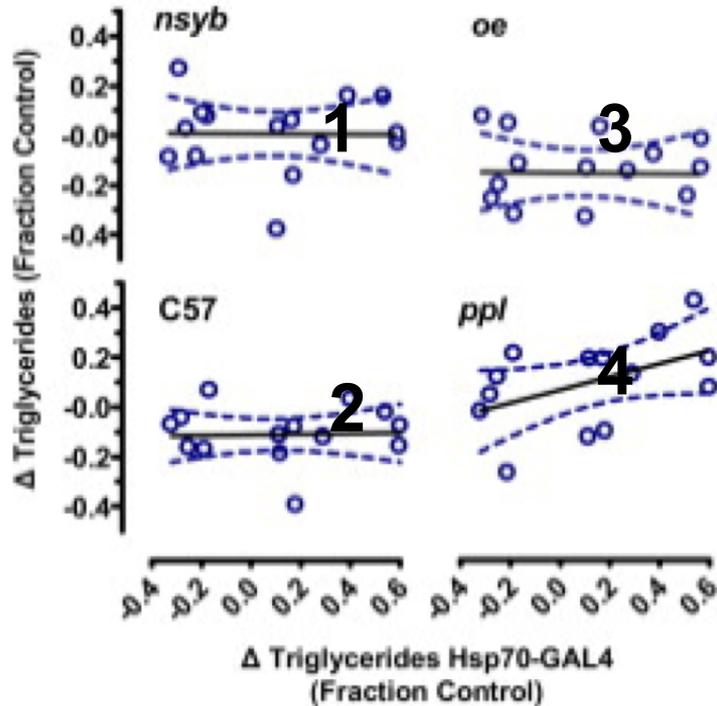
How was the data quantified?

How was the data quantified?

Compound	Marquis	Liebermann	Froehde	Mandelin	Mecke
2C-B	Yellow > Green	Very dark green	Yellow	Green	Yellow
3-MeO-PCP	No colour change	Reddish Brown	No colour change	Green > Green/Brown	Yellow
4-FA	No colour change	Reddish Orange	Faint purple-blue	Pale Blue	No colour change
Amphetamine	Red-Yellow > Brown	Orange	No reaction or Red	Greenish Brown	No colour change
Benzocaine	No colour change	No colour change	No colour change	Light orange-brown	No colour change
Ethylone	Bright Yellow	Greenish Brown	Yellow > Green	Brown	Bright Yellow
Cocaine	No colour change	Yellowish or Orange	No colour change	Very slight darkening	No colour change
Heroin	Violet - Reddish Purple	Black	Purple/Red > Green	Dark Brown	Yellow > Green
Ibuprofen	No colour change	Dark reddish brown	No colour change	Dark Brown	Light brown
Ketamine	No colour change	Light Yellow	No colour change	None or Faint Orange	No colour change
Levamisole	No colour change	Red-orange		Greenish Brown	No colour change
LSD (in liquid)	Olive Black		Yellow Green	Red	Greenish black
MDAI	Orange	Green > Black	Green > Black	Very Dark Brown	Green
MDA	Violet/Purple > Black	Green > Dark Violet	Greenish black	Purple/Blue > Black	Green > Dark Blue
MDMA	Blue > Violet > Black (Maybe hint of green)	Intense Brown - Black	Black with hints of greenish brown	Purple/Blue > Black	Green > Dark Blue
MDPV	Bright Yellow	Yellow > Green	Bright Yellow	Green/Brown	Bright Yellow
Mephedrone	No colour change	Bright Yellow	No colour change	No colour change	No colour change
Methamphetamine	Red-Orange > Brown	Red or Orange	No colour change	Green > Blue	No colour change
Methoxetamine	Pink (slow)	Orange - Brown	Yellow - Green	No colour change	Yellow > Green > Red
Modafinil	Yellow/Orange > Brown	Darkening Orange	Red/orange	Brownish red	Orange > Brown
MPA	Dark Brown	Dark Brown	Light Brown	Reddish Brown	Black
Paracetamol	No colour change	Brownish purple	Pale Blue	Moderate Olive	No colour change
PMA / PMMA	No colour change	Purple - Brown or Orange	Pale Green or Green > Red/Brown	Rust	Olive Green
Procaine	No colour change			Deep Orange	Very light yellow
Vitamin C	Very light yellow	Brown > Purple/Black	Pale Yellow	Pale Blue	Orange (slow)

Colorimetric determination  Triglyceride levels

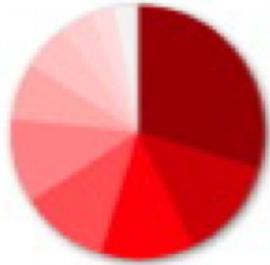
How were candidate genes tested?



Neuronal (*nsyb*-GAL4)
Muscle (*C57*-GAL4)
Oenocyte/Liver (*oe*-GAL4)
Fat-Body (*ppl*-GAL4)

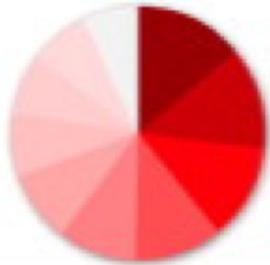
462 RNAi lines crossed to
GAL drivers of **four** different tissue types

What did gene ontology show us?



oenocyte

- neuronal cell fate specification
- regulation of intracellular transport
- imaginal disc lineage restriction
- stalk formation (Insecta)
- female germ-line encapsulation
- reg. of smoothed signaling
- response to organic cyclics
- hematopoiesis
- regulation of organ size
- regulation of mitotic cell cycle



fat-body

- imaginal disc lineage restriction
- stalk formation (Insecta)
- reg. of smoothed signaling
- response to nutrients
- neg. regulation of cell fate
- germ-band shortening
- regulation of organ size
- germ cell migration
- hematopoiesis
- epithelial cell migration

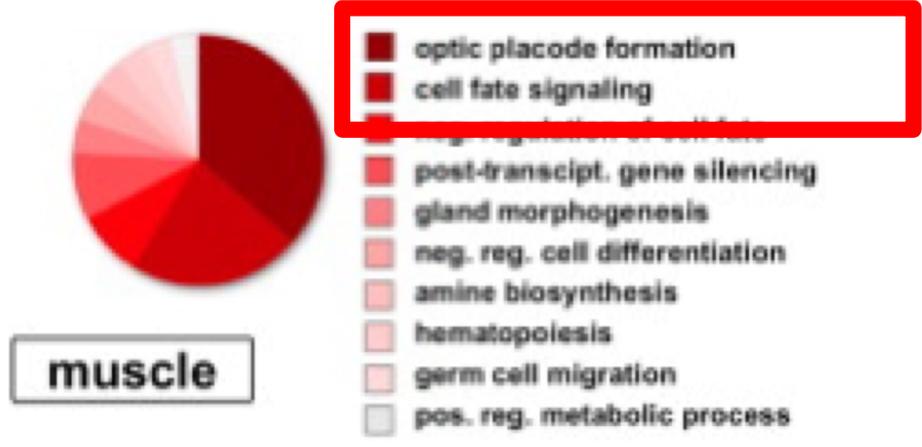
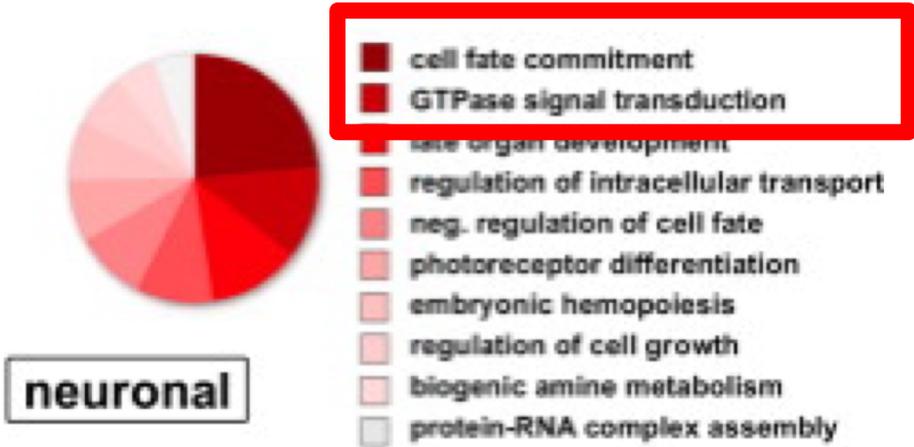
Cell fate

Cell differentiation

& Organ development

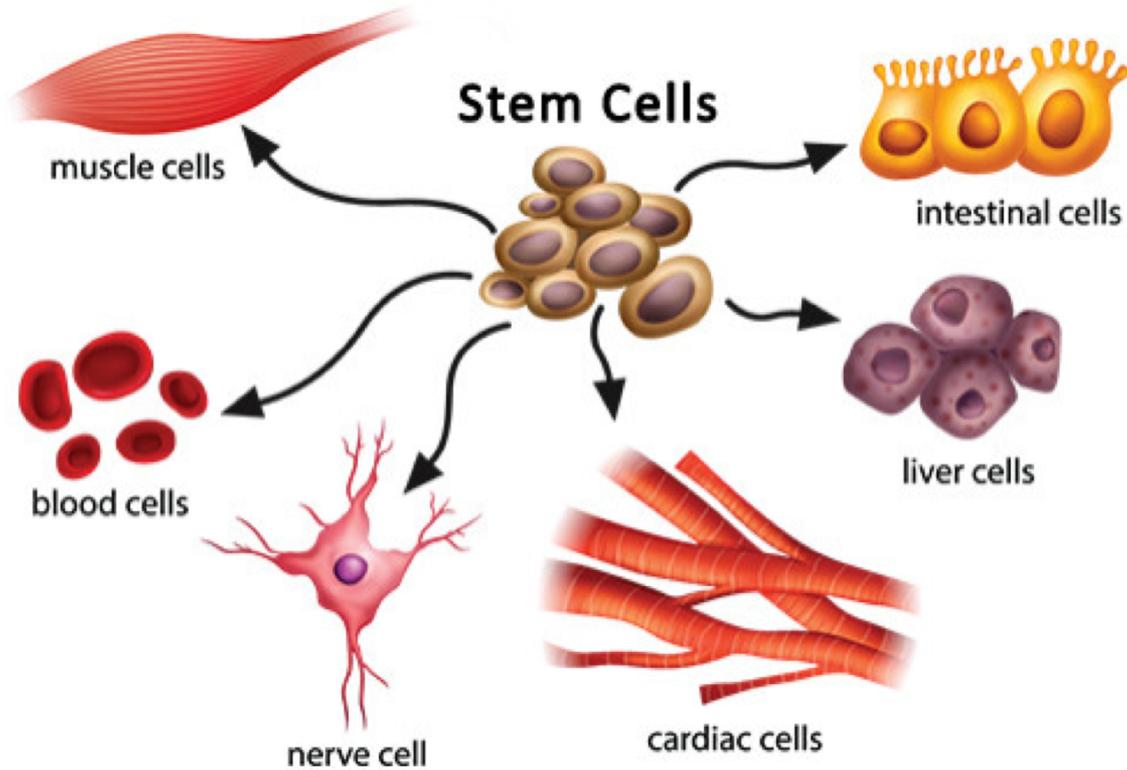
showed strong enrichment

What did gene ontology show us?



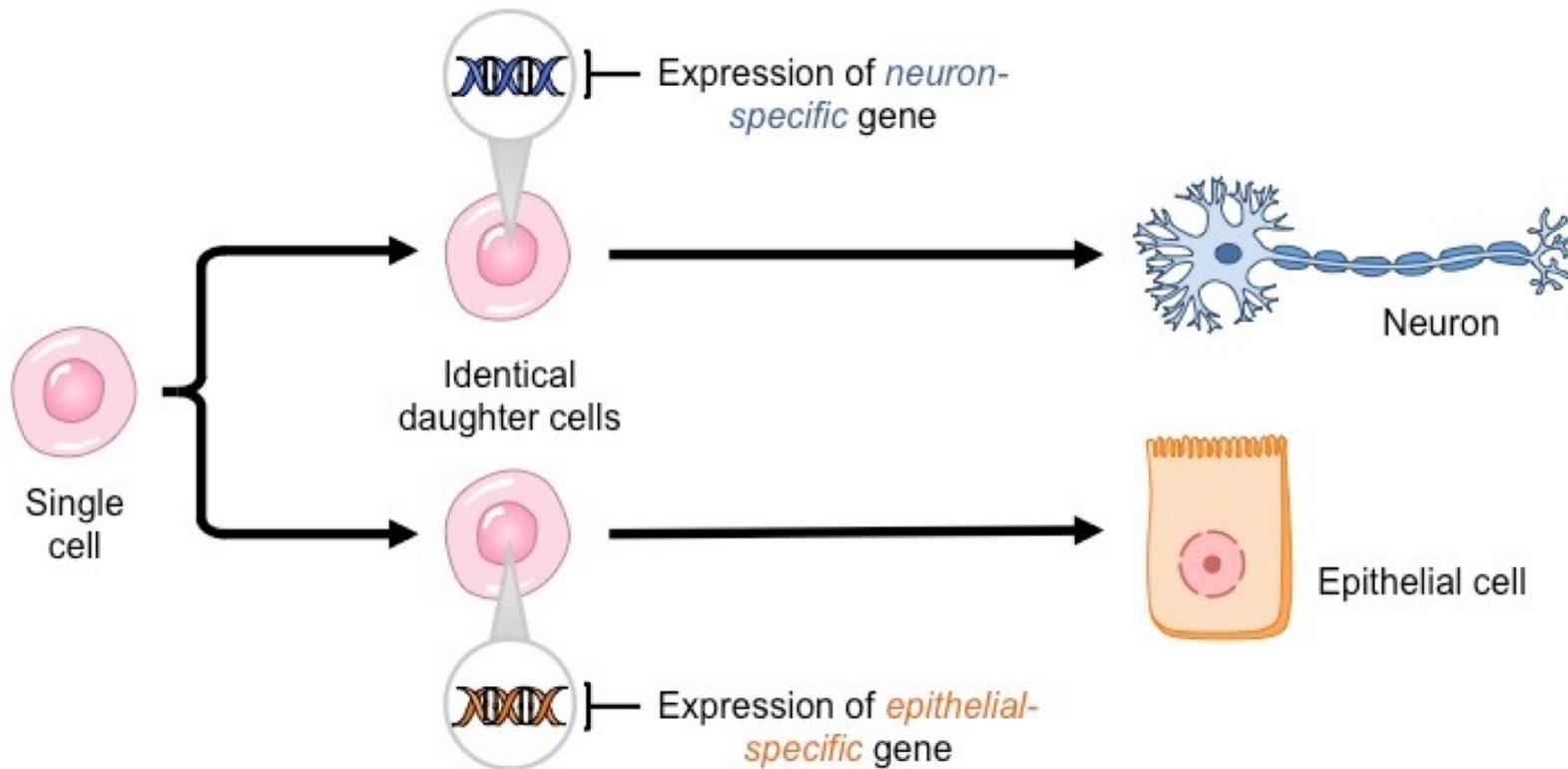
What is cell differentiation?

What is **cell differentiation**?



How embryonic cells become specialized cells

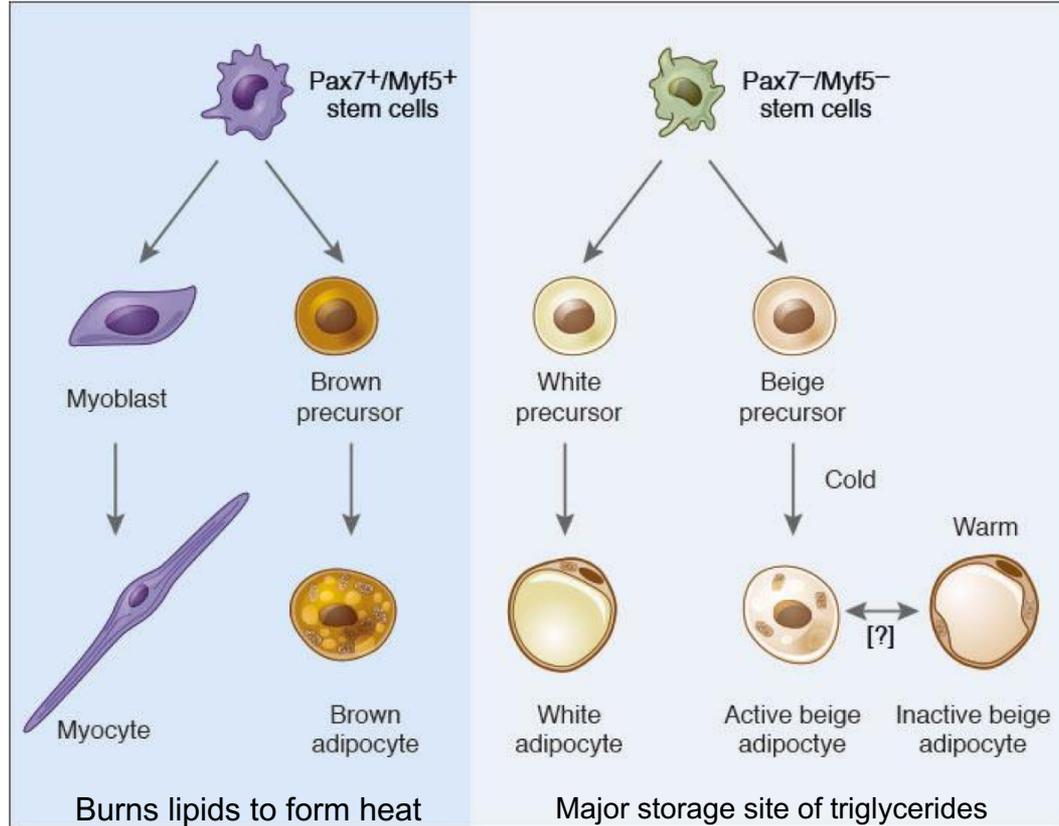
What was the “top hit” obesity candidate gene?



Regulation of **smoothened (Hedgehog) Signaling**

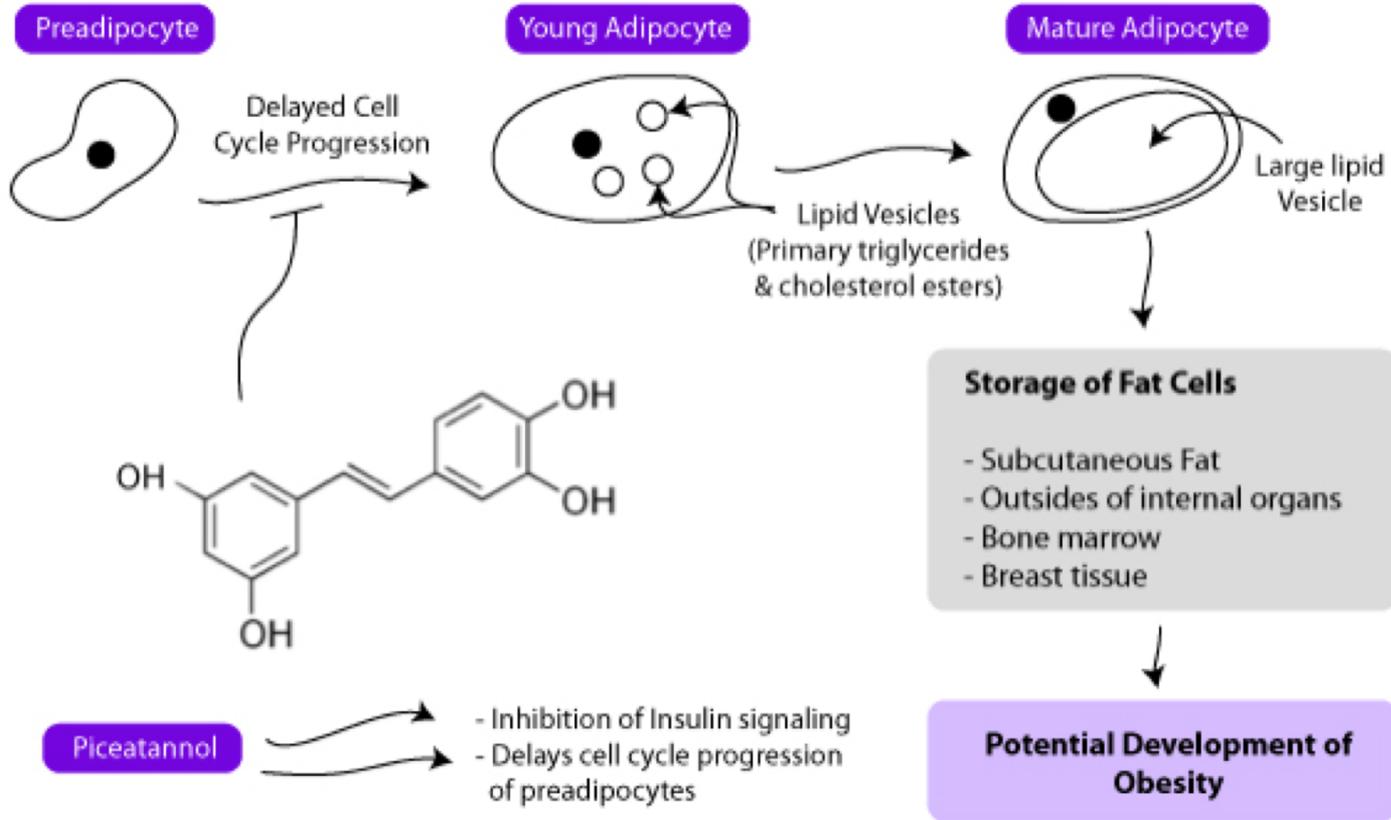
What type of tissue does *Hedgehog* regulate?

**Brown
Adipocyte
Tissue
(BAT)**



**White
Adipocyte
Tissue
(WAT)**

What is adipogenesis?



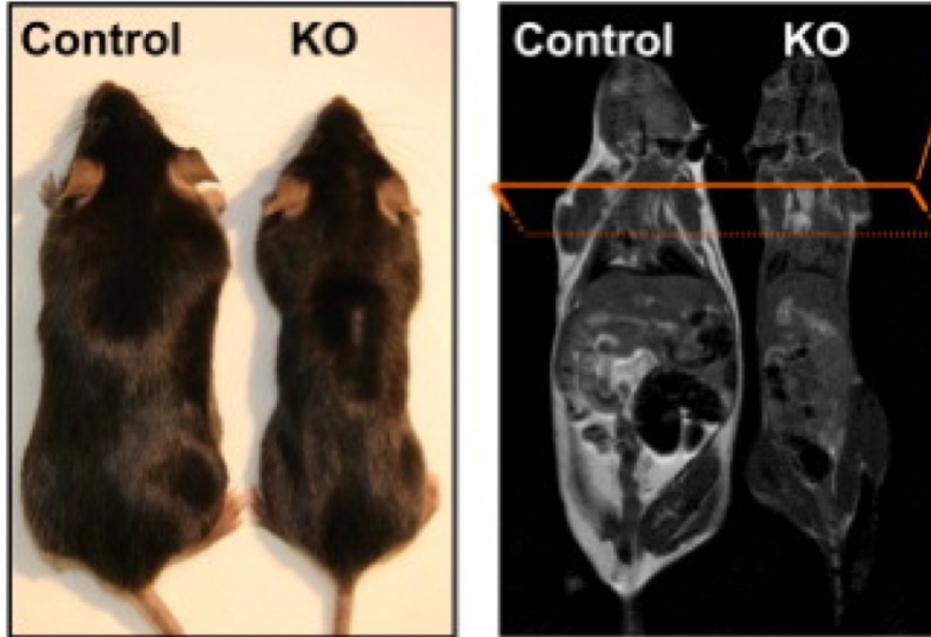
Preadipocytes



Adipocytes

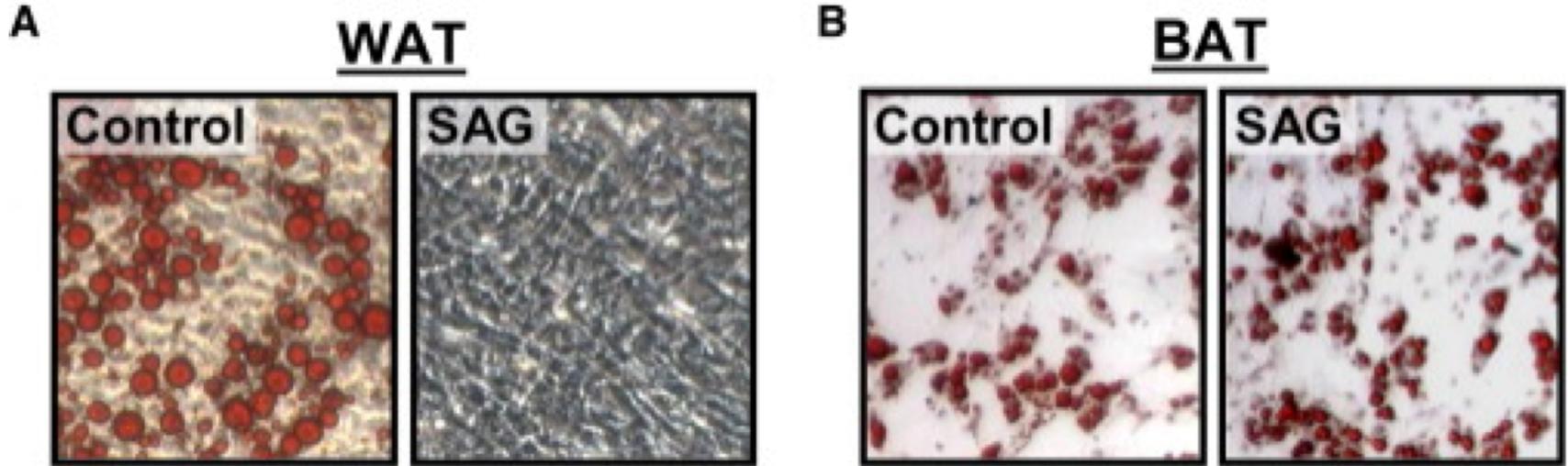
How was the Hedgehog pathway studied?

Fat-specific Hedgehog-activation mutant mice used

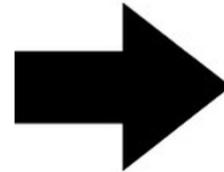


Sufu knock-out mice crossed to
adipose-deleting *cre* transgenic mice

What was the next step?

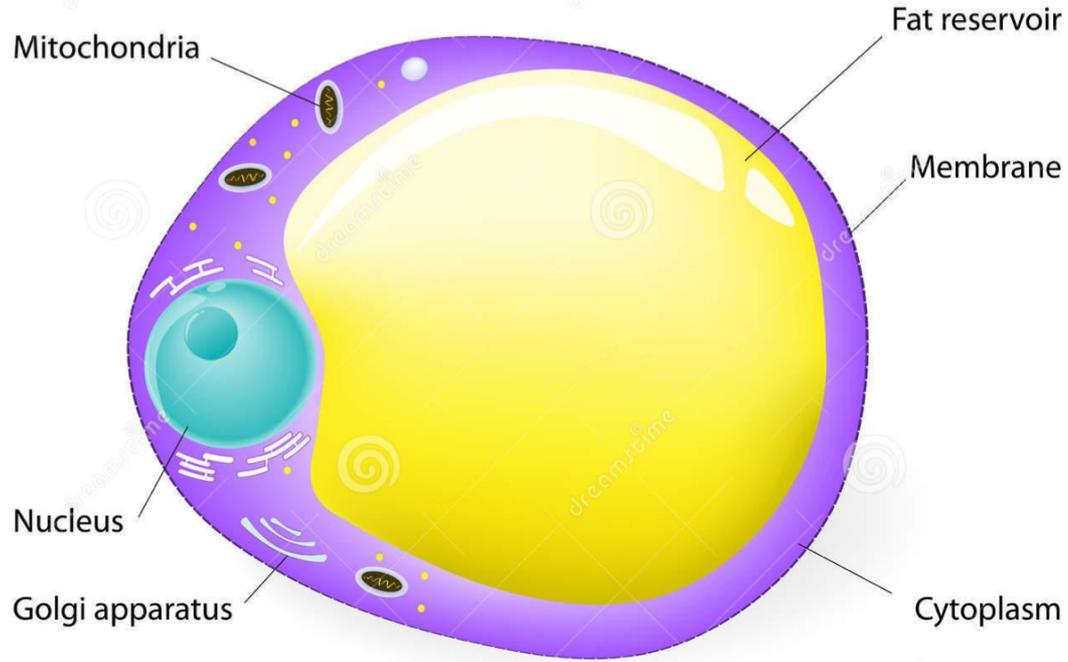


Stimulating
Smoothened Agonist (SAG),
an activator of Hedgehog

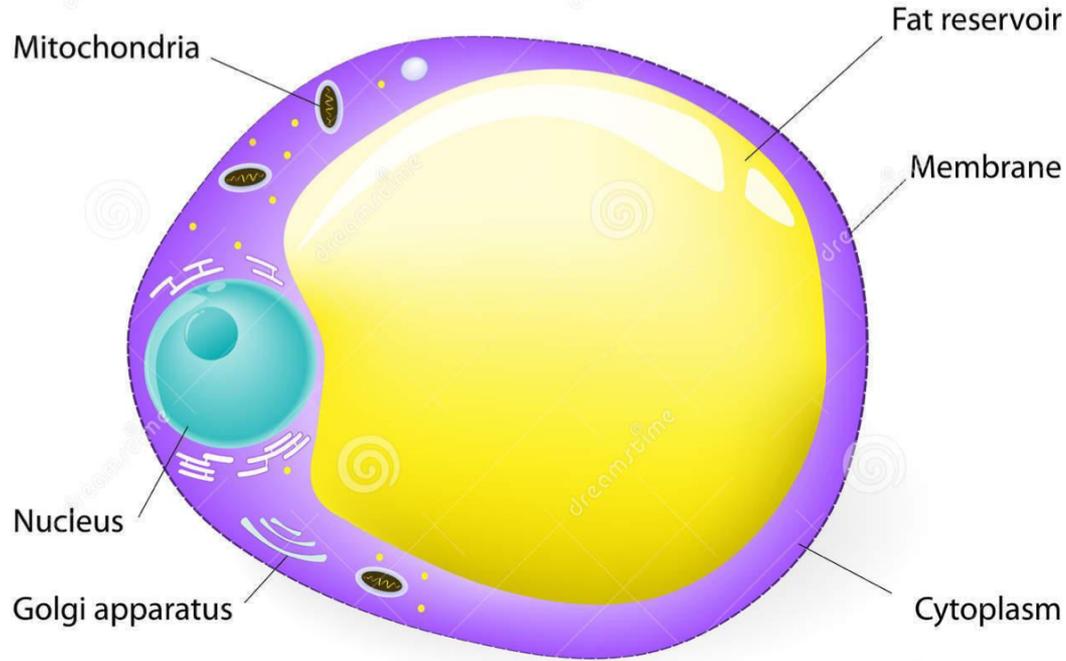


Blocked
adipogenesis
in WAT

What are adipogenic factors?



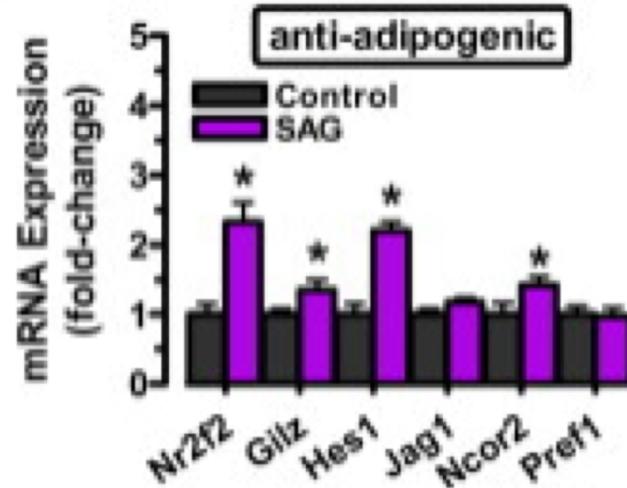
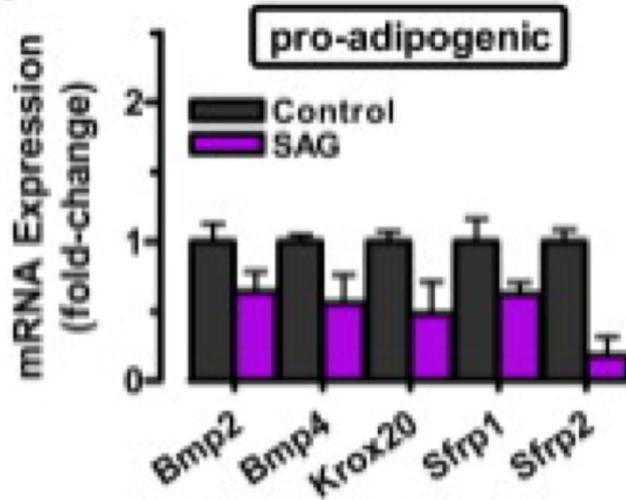
What are adipogenic factors?



Transcription factors crucial to adipogenesis

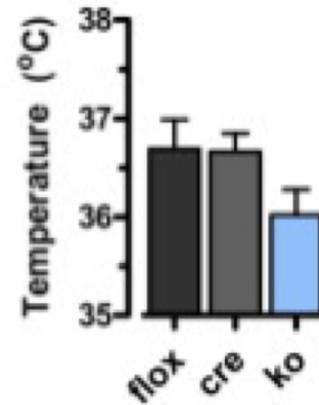
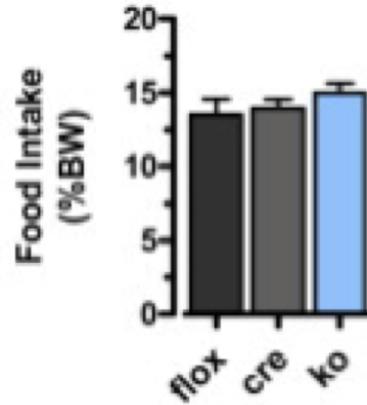
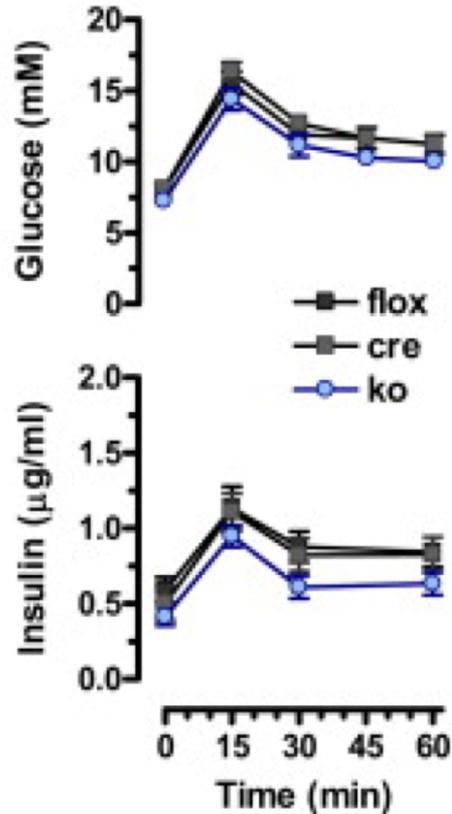
What is the effect of Hedgehog activation on adipogenic factors?

What is the effect of Hedgehog activation on adipogenic factors?

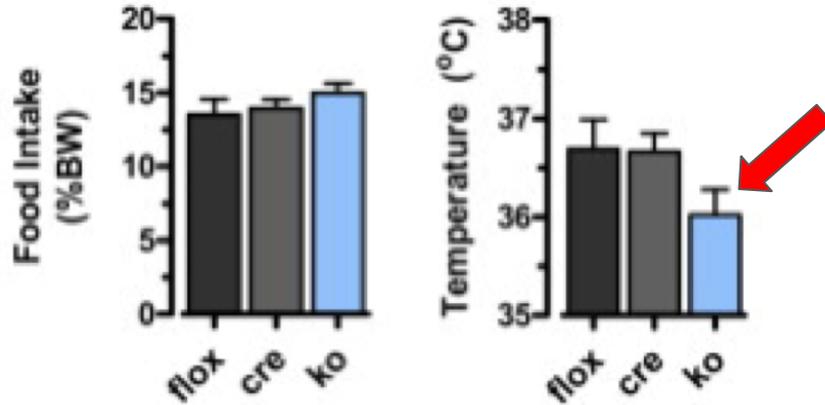
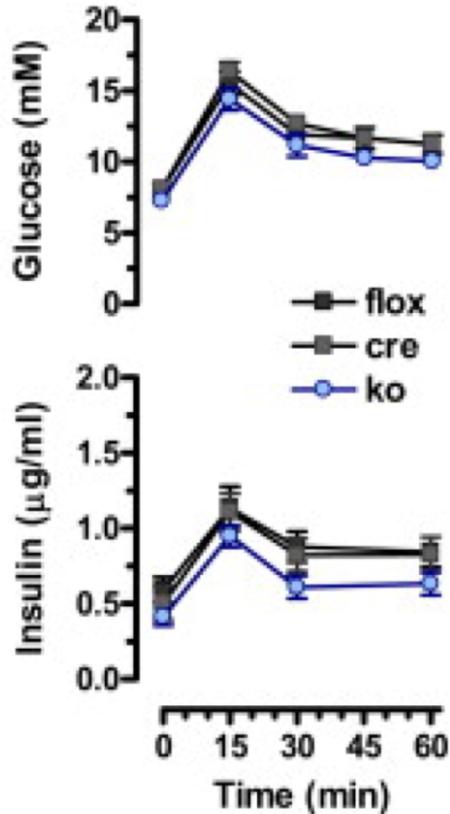


Upregulation of anti-adipogenic factors
Downregulation of pro-adipogenic factors

Are there metabolic consequences to the loss of fat tissue?



Are there metabolic consequences to the loss of fat tissue?



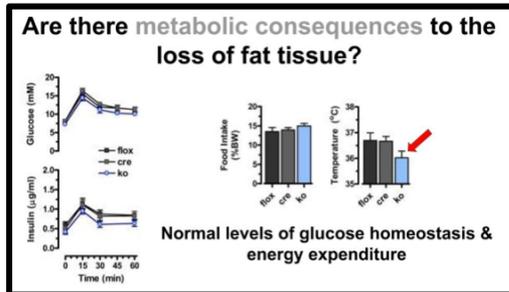
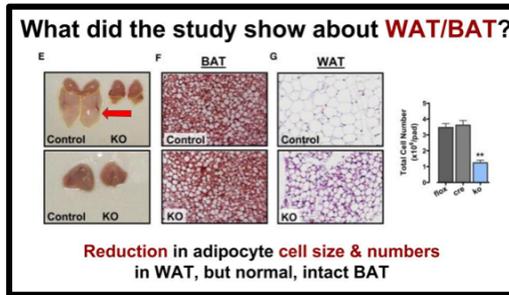
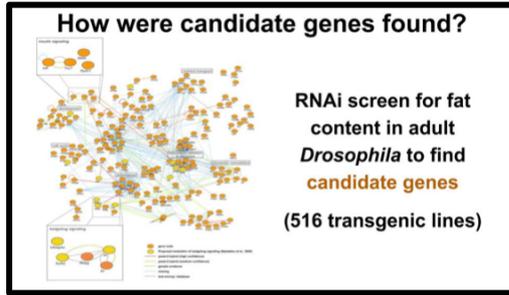
Normal levels of glucose homeostasis & energy expenditure

Summary

RNAi screening used to identify **candidate** obesity genes

Activation of hedgehog pathway **inhibits WAT cell differentiation** through modulation of adipogenic factors

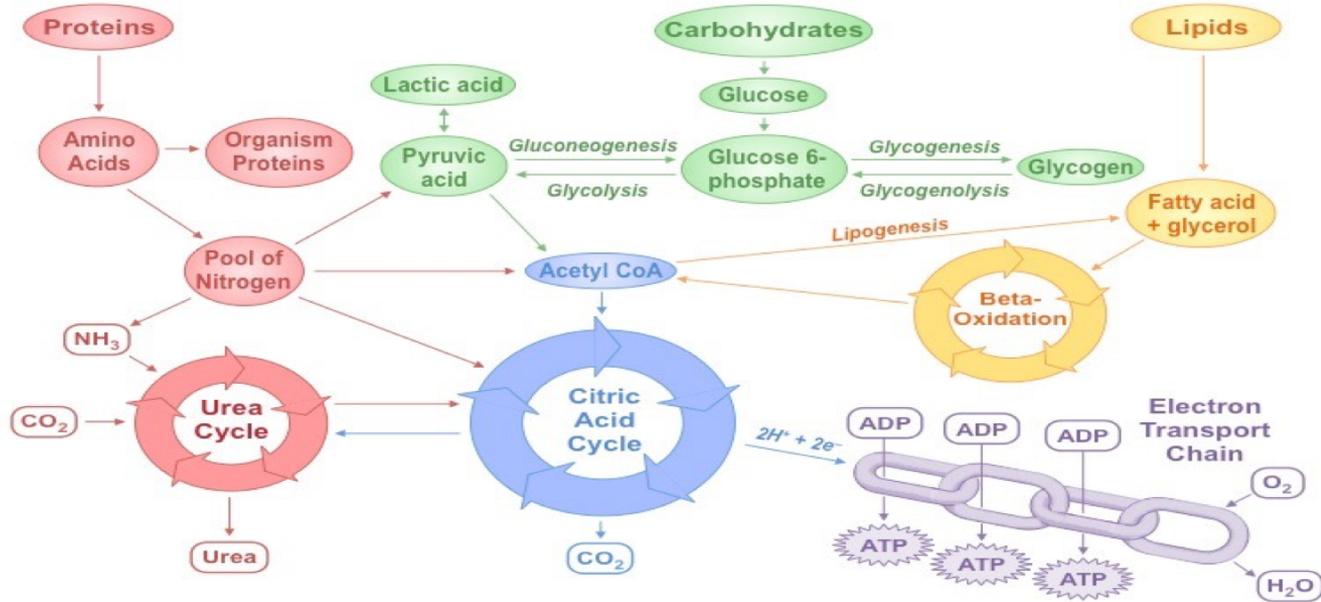
Sufu knockout mice exhibit **normal** glucose tolerance & energy expenditure



Why is this study **important**?



Why is this study important?



Can study **adipocyte regulation** in mammals/humans

Important for metabolism, energy homeostasis & other contributing pathways/factors to obesity

ANY
QUESTIONS
?

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