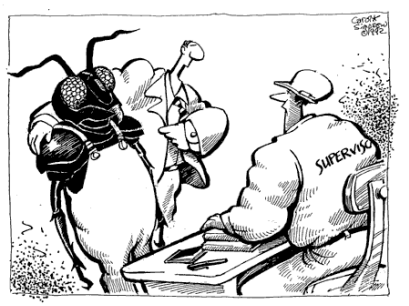
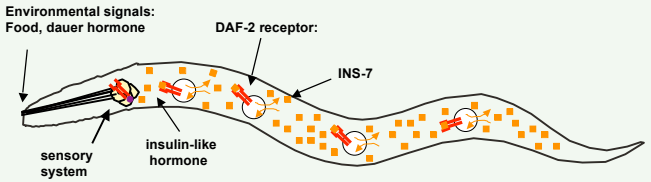
Insulin-like signaling pathway: flies and mammals



"His entire adult lifespan is exactly eight hours. He won't be around long enough to need benefits."

A&S300-002 Jim Lund

Insulin-like signaling responds to environmental signals



This feedback loop may allow all the cells to make the same developmental or lifespan decision

Drosophila insulin-like signaling pathway (ISP)

- Genes in this pathway were first investigated for effects on growth and size.
- ISP also affects blood sugar levels in the fly.
- Fly has five insulin-like proteins.
  - Expressed strongly in small clusters of cells (IPCs) in the brain.
  - Ablation of IPCs causes retarded growth and higher carbohydrate levels (Rulifson et al., 2002).

Drosophila insulin-like signaling pathway (ISP)

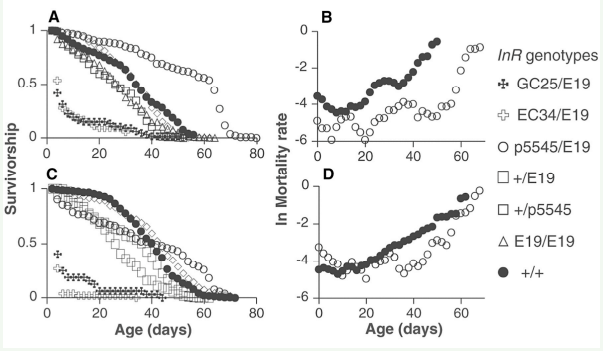
- The gene InR is an insulin-like receptor in fruit flies.
- It is homologous to insulin receptors in mammals and to *daf-2* in worms.
- Studied InR gene variants (alleles) in flies.

(Tatar et al., 2001)

InR: various allele combinations produce different results

- Some had a reduced survival rate
- Females in one type extended life span by 85%
- Males followed the female pattern in most cases
- Not all the InR alleles extend longevity because the gene is highly variable.
- Some alleles produced developmental defects that carry over into adults.

InR: various allele combinations produce different results



InR^{EC34}/InR^{E19}, InR^{GC25}/InR^{E19}, $InR^{E19}/InR^{E1}9$, and $+/InR^{p554}$

Females: A, B Males: C, D

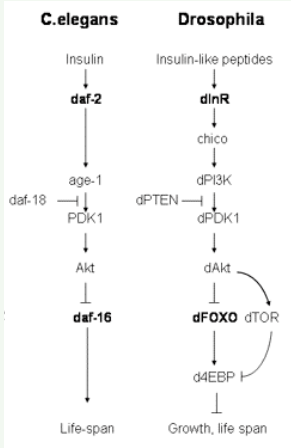
Fly insulin-like signaling pathway

- Fly homolog of *daf-16*: **dFOXO**.
- Forkhead box DNA binding domain amino acid identity is between 74 and 86 percent.
- Akt phosphorylation sites are also well conserved
- dFOXO heterozygotes supress InR lifespan extension.

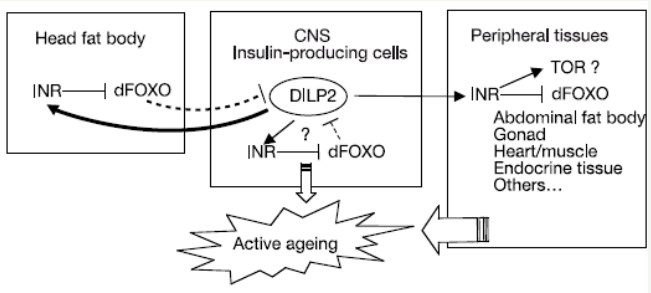
Fly insulin-like signaling pathway

- Fly has four homologs of the PI3K *age-1*.
- Each controls different cellular processes.
- Increased signaling complexity in the fly relative to the worm.
- More complicated in human, 16 PIK3 genes.

Fly and worm insulin-like signaling pathways



Drosophila ISP regulation of lifespan



Nature **429**: 562-66, 2004

Mammal insulin-like signaling pathway

- In vertebrates, the insulin receptor regulates glucose metabolism, while IGF-1R promotes growth.
- IGF-1R is activated by its ligand IGF-1, which is secreted in response to growth hormone.
- Pathway more complicated: more tissue specific signaling and regulation.
  - Multiple homologs, some specific to certain somatic tissues.
  - Genetic investigation is more complicated.

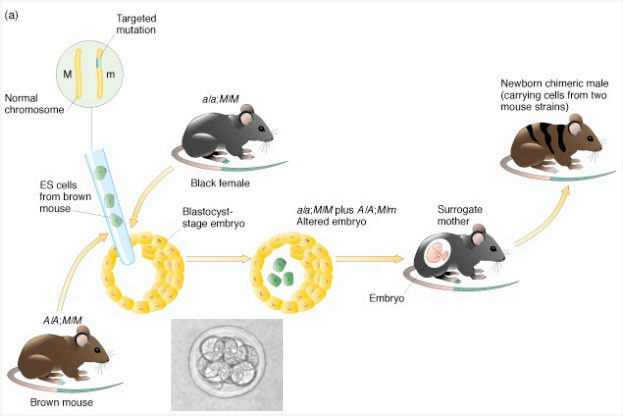
Mammal insulin-like signaling pathway

- In mice, inactivation of the growth hormone receptor decreases circulating IGF-1, impairs growth development, and increases lifespan.
- Calorie restriction, the only intervention demonstrated to reliably and consistently increase mammalian lifespan, always reduces circulating IGF-1.

Mammal gene knock-out technology

- Recall that most organisms have two copies of each gene, one inherited from each parent.
- Using genetic engineering methods, it is possible to delete or otherwise alter one or both copies of a gene, so that the animal has either one or no working copy of the gene.
- A mouse altered in this way is called a "knock-out" mouse.

Mammal gene knock-out technology



Mammal insulin-like signaling pathway

- When both copies are knocked out, it is called a homozygous null mutant, or a double knock-out.
- An IGF-1R double knock-out is annotated *Igf1r* -/-
- When one copy of IGF-1R is knocked out, it is called a single knock-out, annotated *Igf1r* +/-.
- Horzenberger created *Igf1r* -/- and *Igf1r* +/- mice. The double knock-out *Igf1r* -/- mice did not survive. The single knock-out *Igf1r* +/- mice survived.

Igf1 knock-out mice

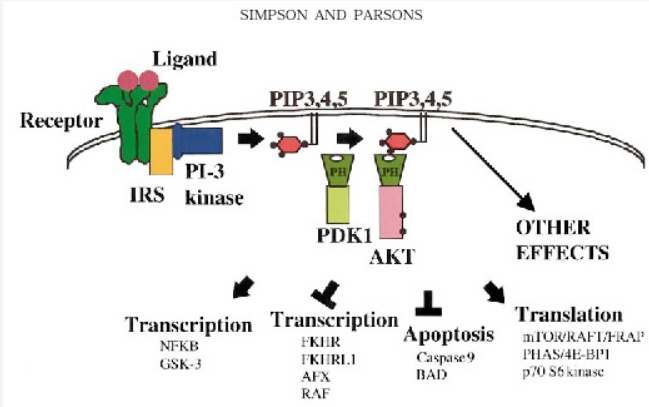
- The single knock-out Igf1r+/- mice lived an average of 26% longer than wild-type mice.
- Female Igf1r+/- mice lived an average of 33% longer than wild-type,
- Male Igf1r+/- mice lived an average of 16% longer.

ISP in Rats

- Rats with reduced GH/IGF-1 levels.
- 40% reduction in IGF-1 levels produces a 9-13% lifespan extension.

(Shimokawa et al., 2003)

Mammal ISP: cellular processes controlled



Centenarian genetics: human INSR

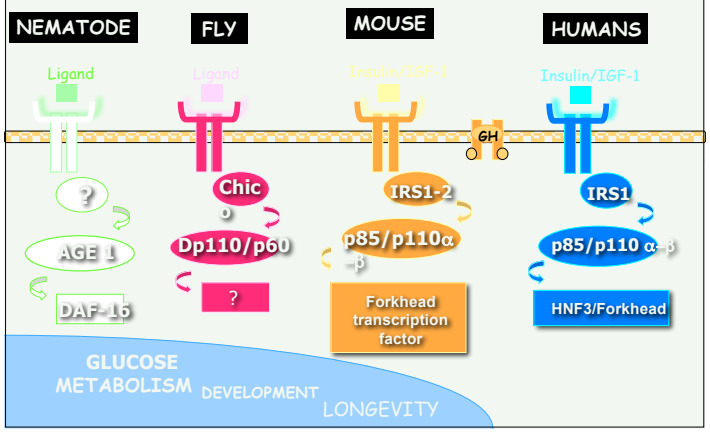
INSR

•Study of 122 Japanese semisupercentenarians (older than 105) with 122 healthy younger controls.

•One INSR haplotype, which was comprised of 2 SNPs in linkage disequilibrium, was more frequent in semisupercentenarians than in younger controls.

Kojima et al., 2004

Insulin/IGF-1 Signaling Pathway: Human Homologies With Nematode, Flies And Mice



Mammalian ISP

•Mouse mutants with reduced insulin signaling live longer.
•Mouse IGF-1 receptor mutant heterozygotes (ie. reduced IGF-1 receptors).

•Dog breeds with low levels of IGF-1 live longer

•Caloric restriction reduces insulin and IGF-1 (increases mammal longevity)

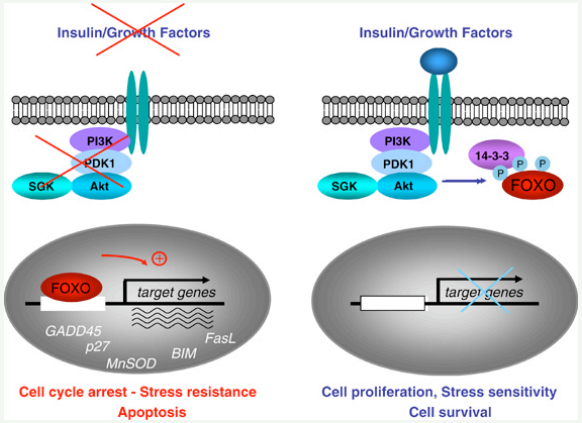
Mammalian Models

- Long-lived mice (like the worms) have been characterized by a deficiency in growth hormone and IGF-1.
- Tissue-specific inactivation of the insulin receptor has been experimentally effective
  - Fat cells in mice = 20% increase.
  - Partial receptor inactivation also effective in mice .
  - Deletion of the p66^{shc} protein in mice results in a 30% increase in longevity…these mice can better withstand oxidative stress.
- Also, cells from these animals have lower levels of oxidants.
- P66^{shc} appears to regulate the mammalian Forkhead-family member counterpart of DAF-16.

Human ISP

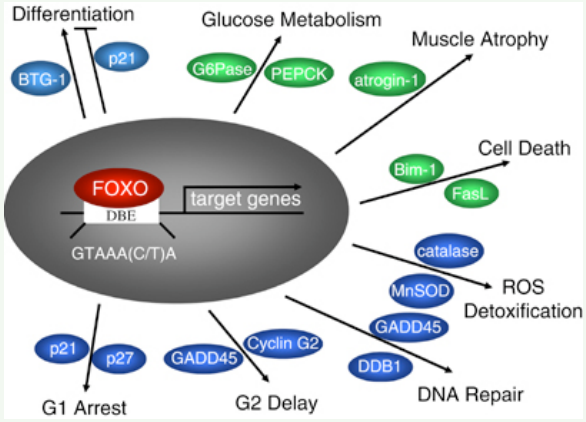
- 7 human homologs of *daf-16*, the Forkhead family transcription factor.
  - Called FOXOs or FKHRs.
- Several FOXOs are tumor suppressors, as are AKT and PTEN.
- Activation of FOXOs lead to:
  - cell cycle arrest, stress resistance, or apoptosis.

Human ISP



Greer and Brunet, 2005

Functions of human FOXOs



Greer and Brunet, 2005

Insulin-like signaling pathway (ISP)