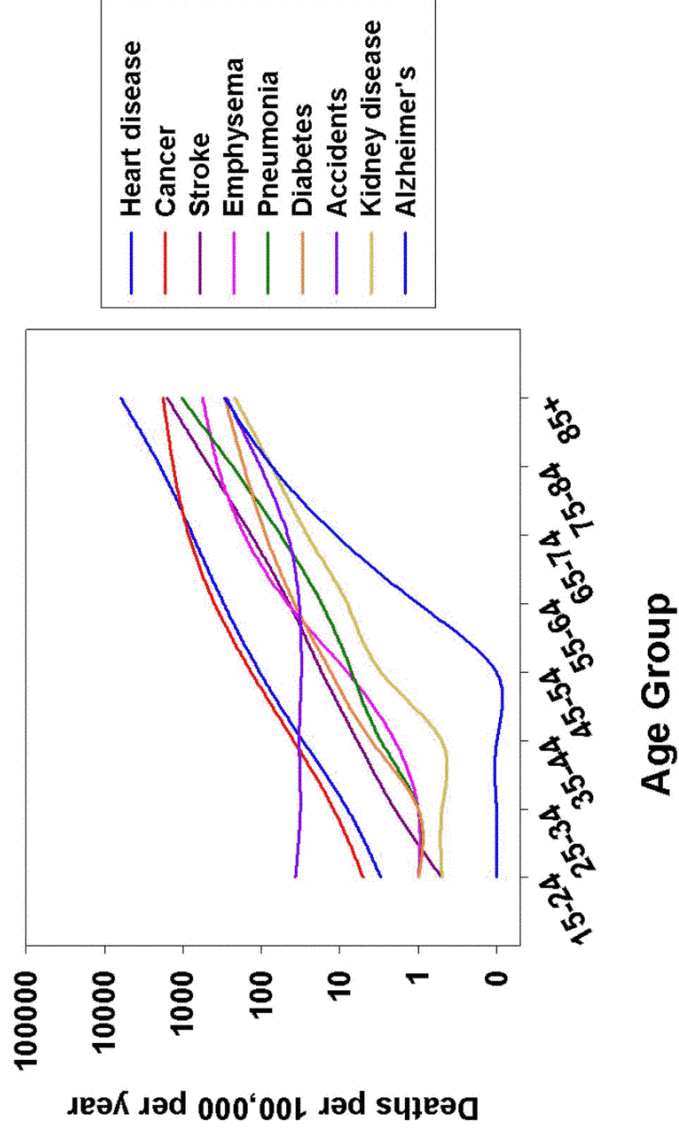
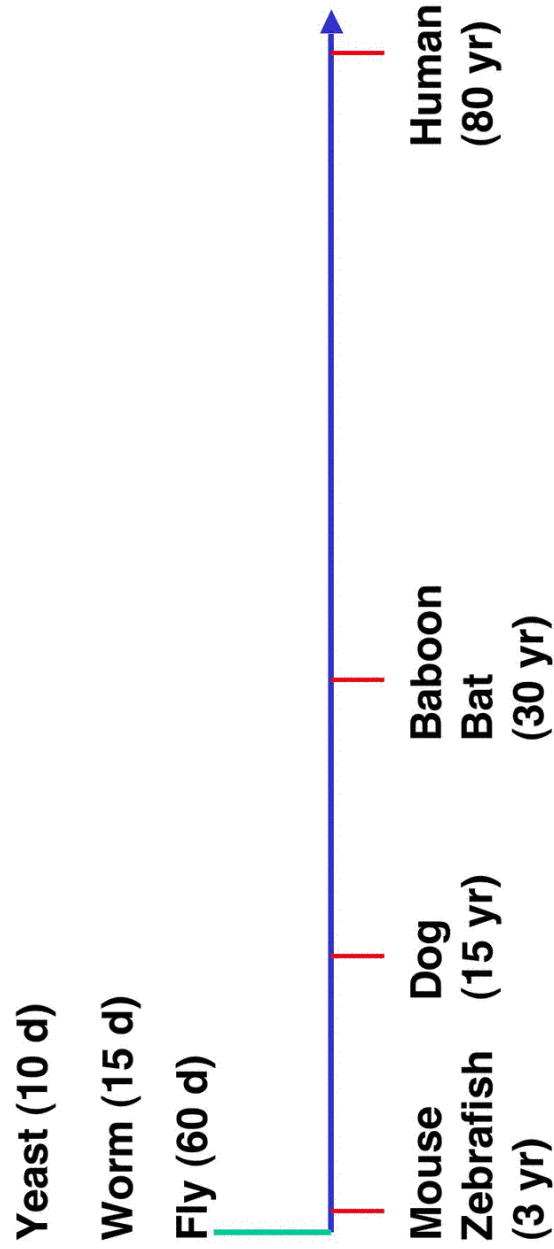


## Aging and age-related diseases

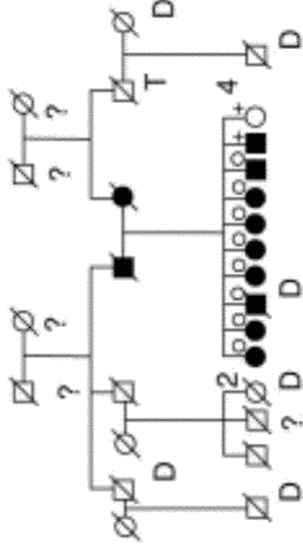


## Organismal Longevity



## Genetic control of lifespan

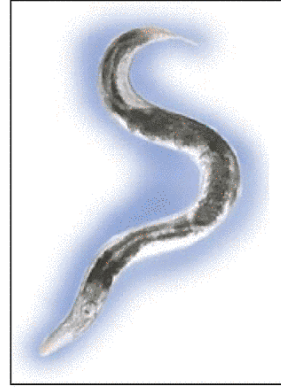
### Studies in families with exceptional longevity



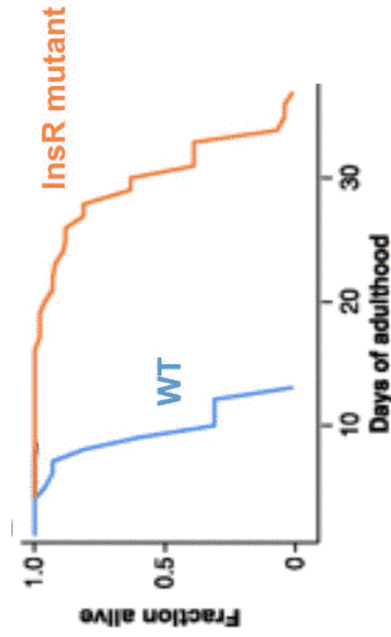
#### Clusters of genes

- Chromosome 4
- Chromosome 19

## Longevity mutants in *C. elegans*

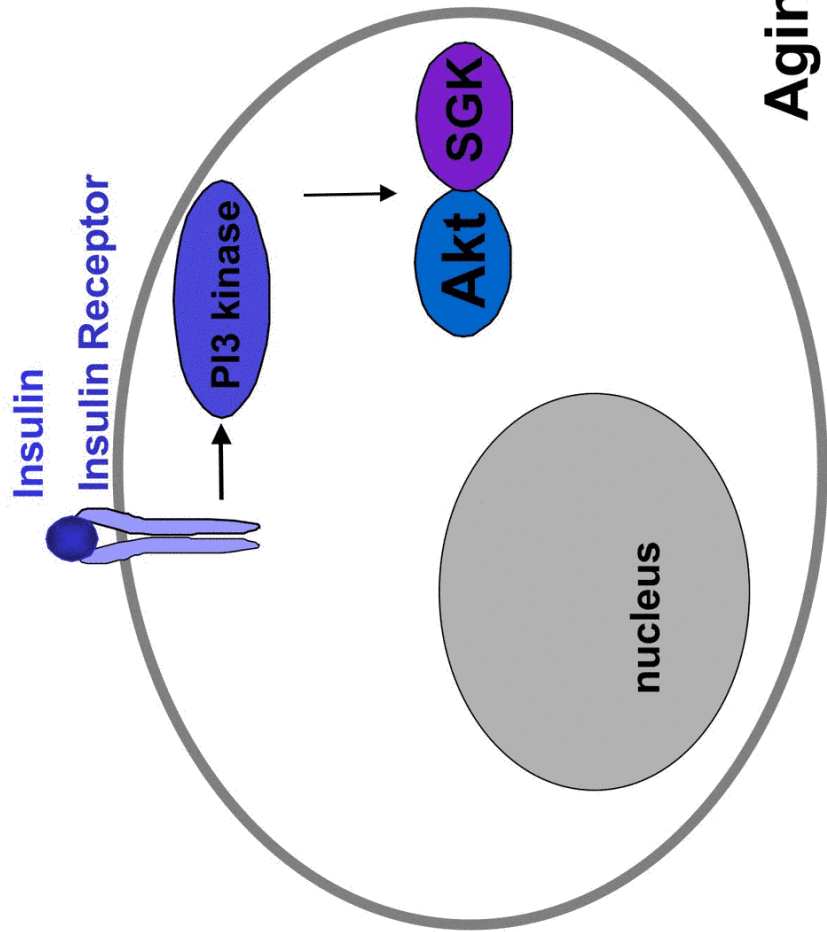


*C. elegans*



Wolkow, Science 1999

# The Insulin signaling pathway

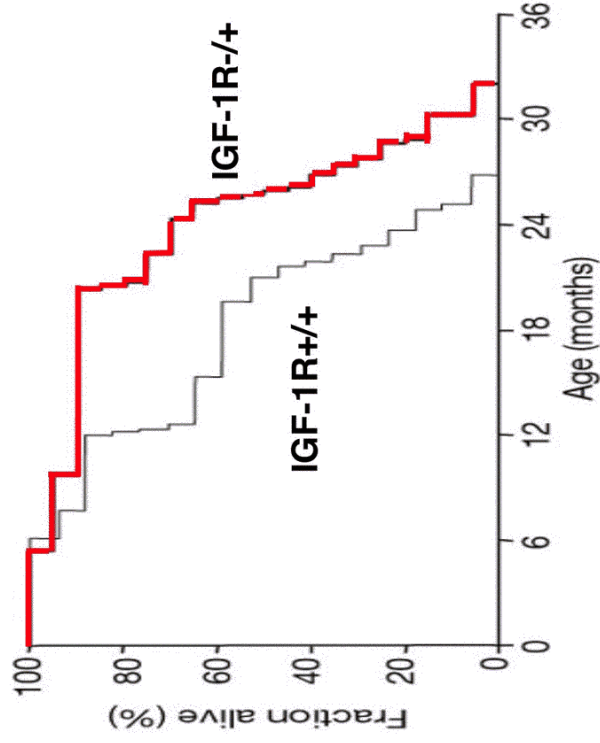


Aging

## Mutations in single genes affect longevity

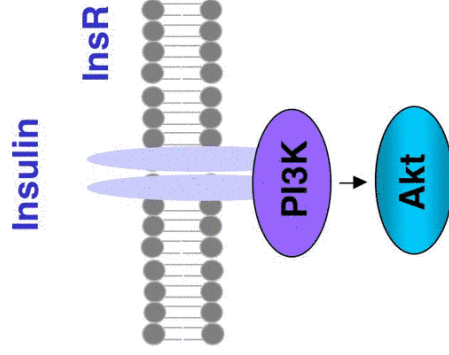
Organism	Gene	Lifespan of mutants
Yeast	<b>Akt</b>	↗
C. elegans	<b>Insulin receptor</b>	↗
	<b>PI3 kinase</b>	↗
	<b>SGK</b>	↗
Drosophila	<b>Insulin receptor</b>	↗

## Extended longevity of mice that are mutant for the IGF-1 receptor



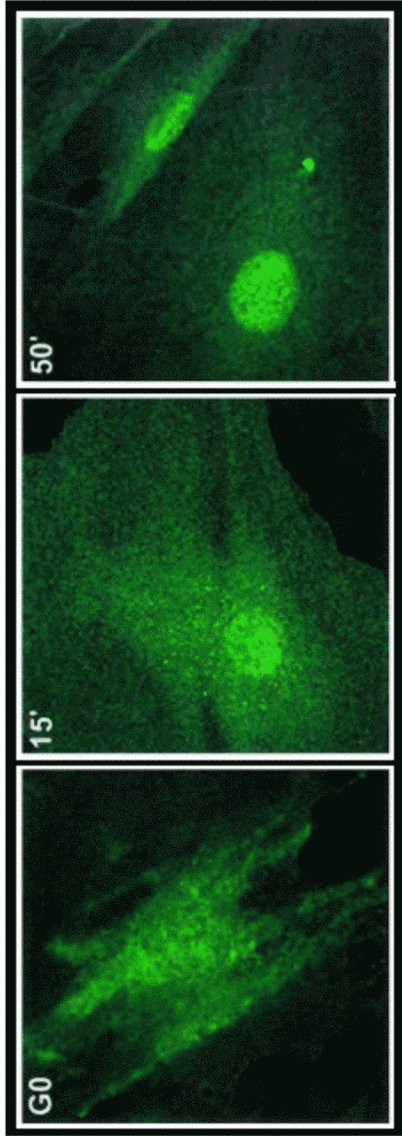
Resistant to oxidative stress

## Importance of the insuling signaling pathway



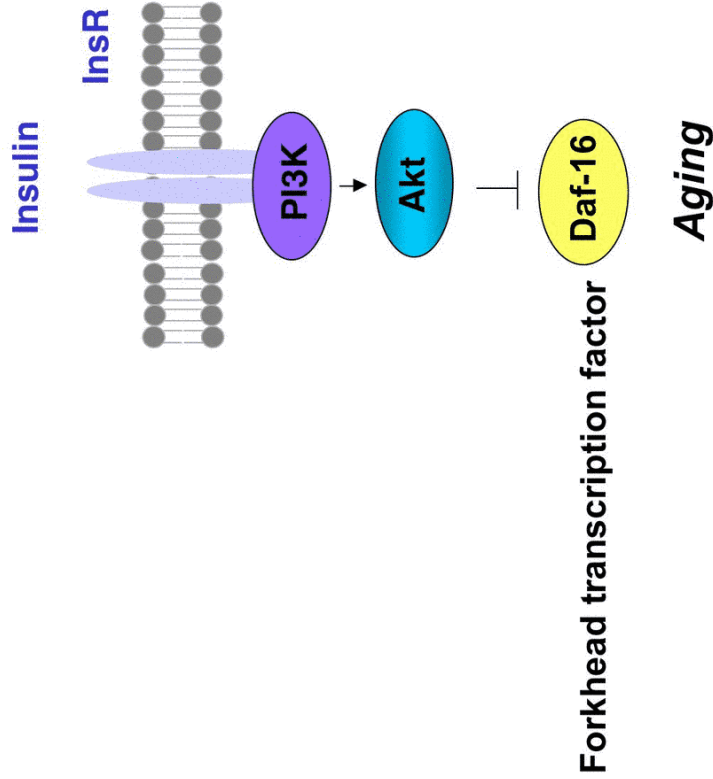
Aging

## Subcellular localization of Akt



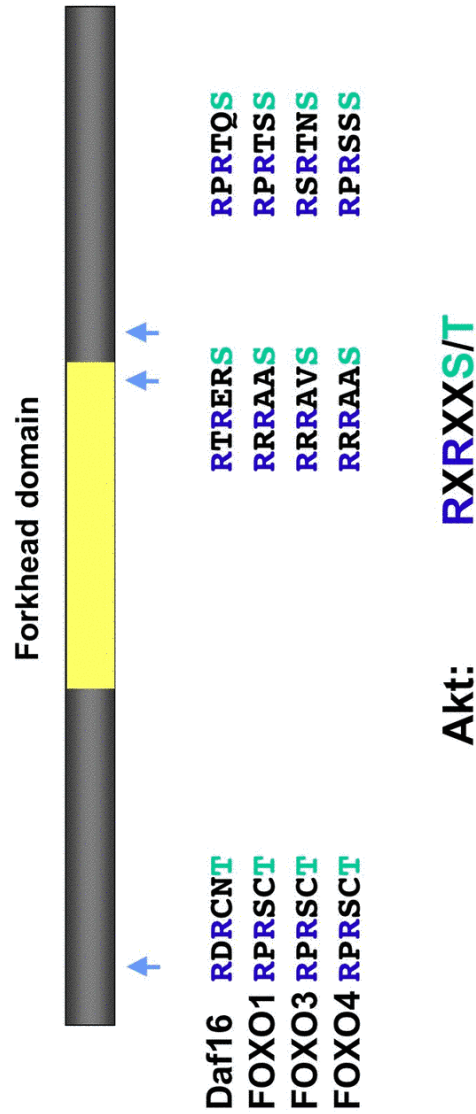
(B.A. Hemmings)

## Forkhead transcription factors of the FOXO family

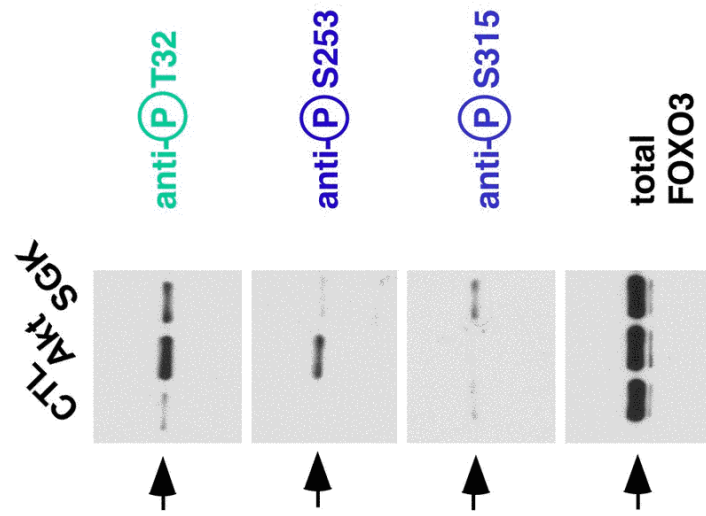


## Daf16 homologues in Human

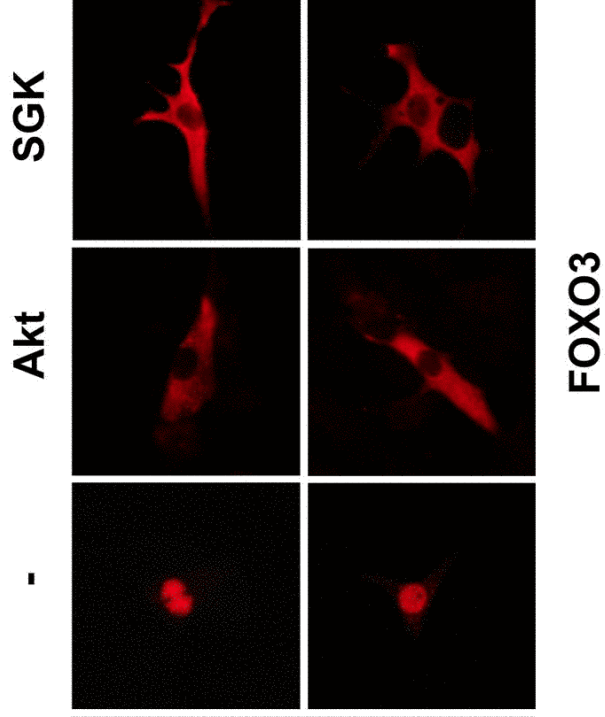
FOXO1: rhabdomyosarcomas  
 FOXO3: acute myeloid leukemia  
 FOXO4: acute myeloid leukemia



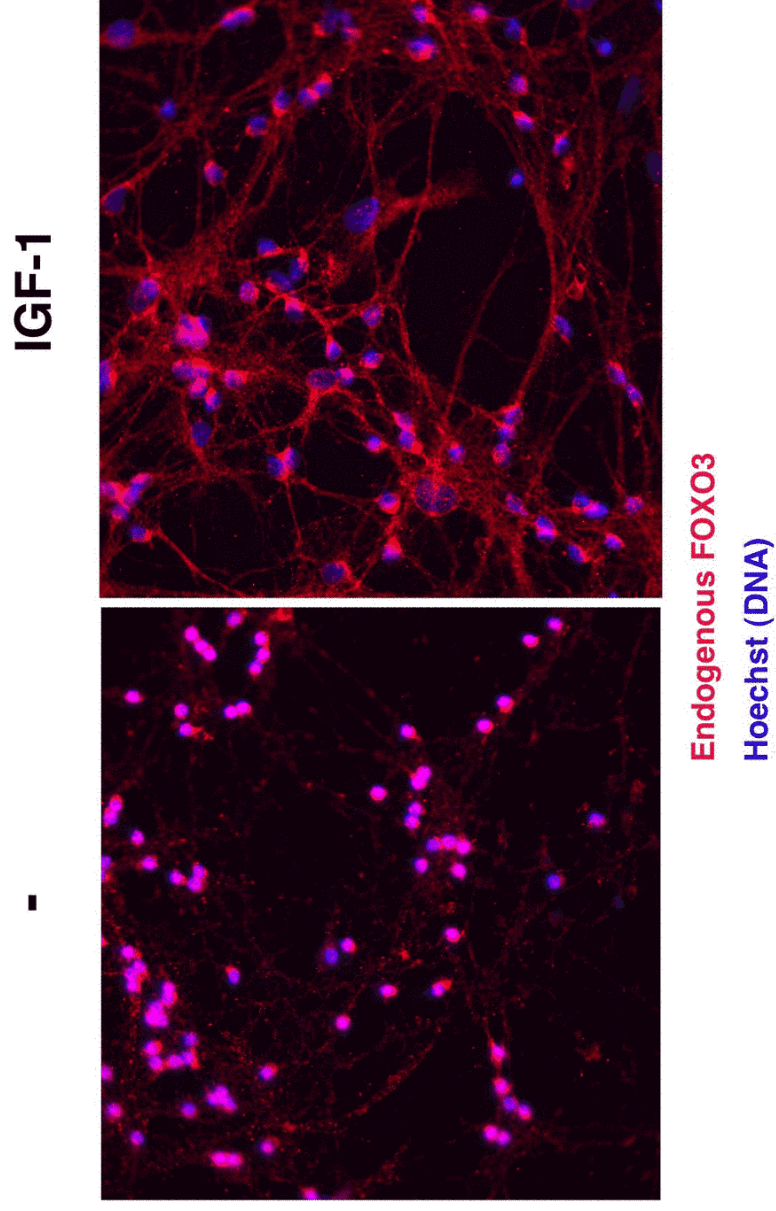
## Akt and SGK phosphorylate FOXO3 within cells



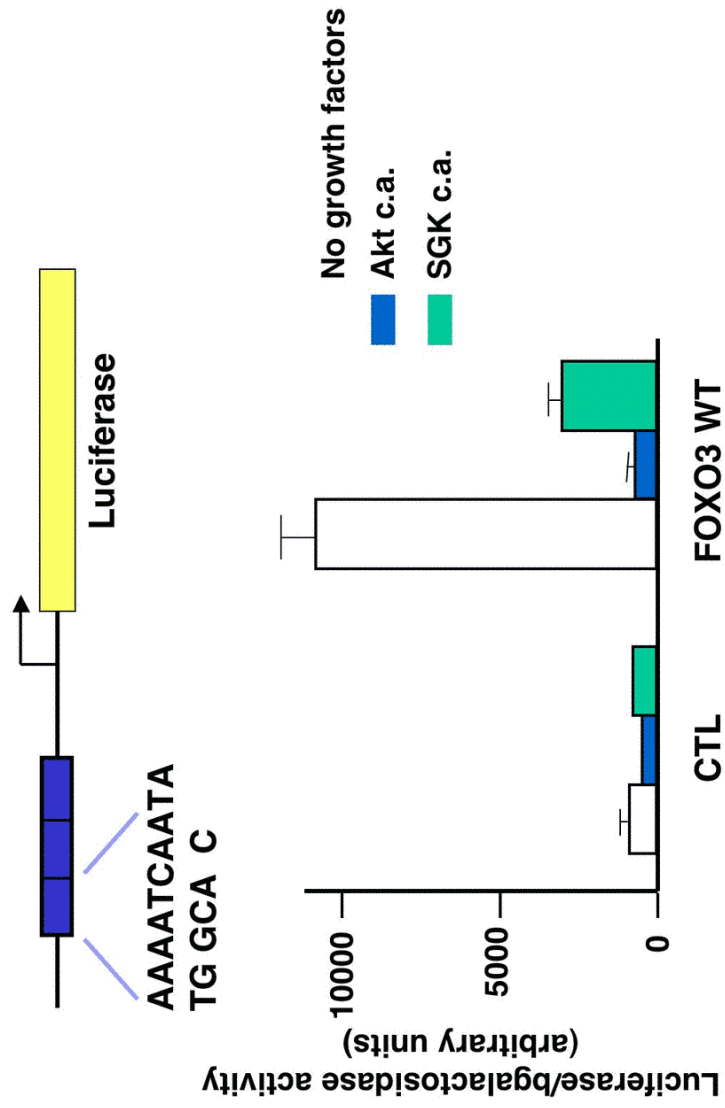
### Akt and SGK promote FOXO3 relocalization in the cytoplasm



### Localization of FOXO3 in cerebellar granule neurons

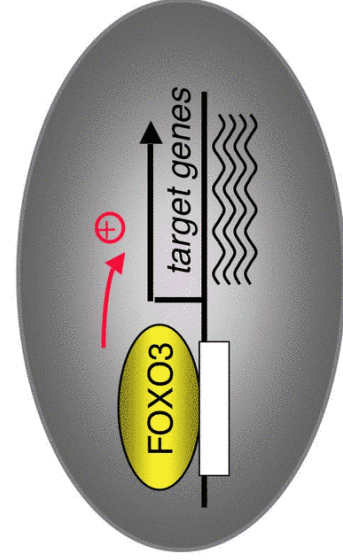


## FOXO3 transcriptional activity



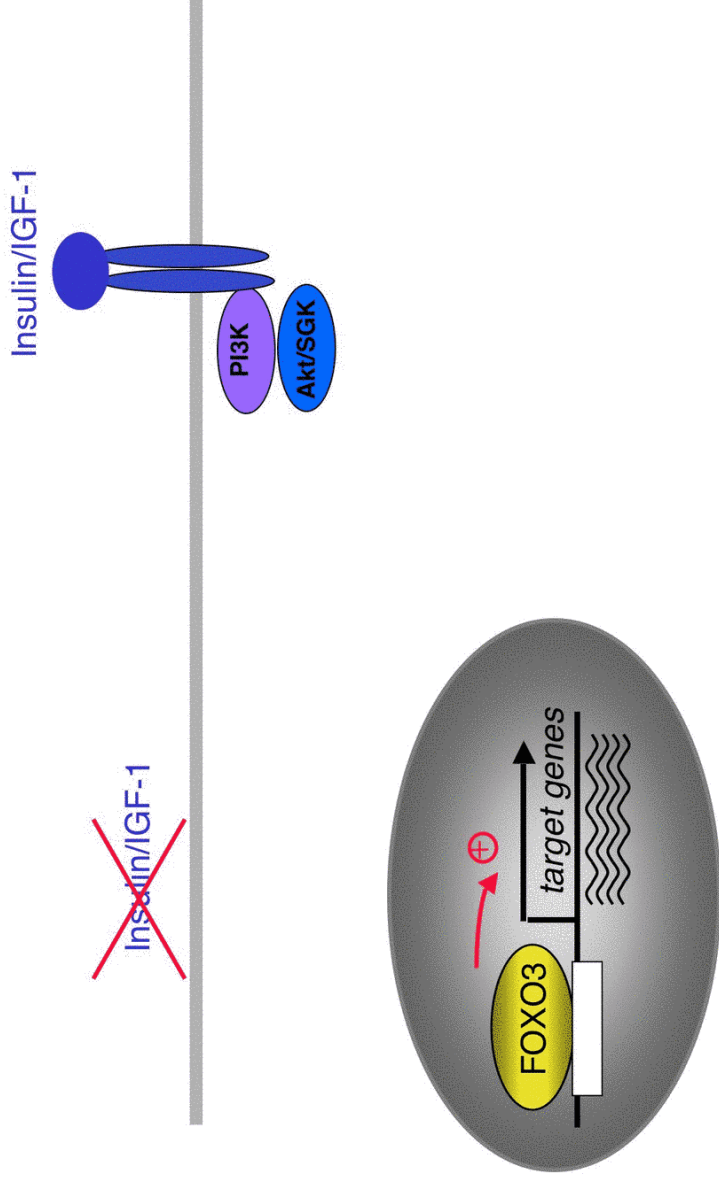
## Regulation of FOXO TF in mammalian cells

~~Insulin/IGF-1~~

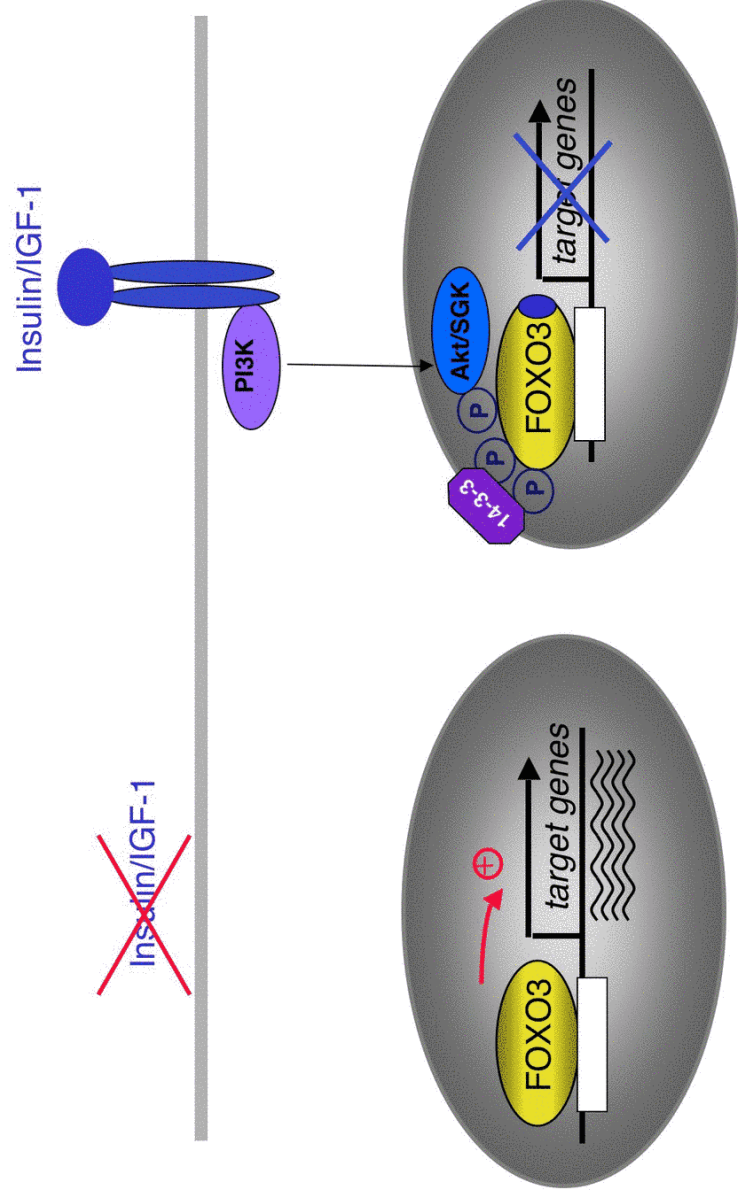




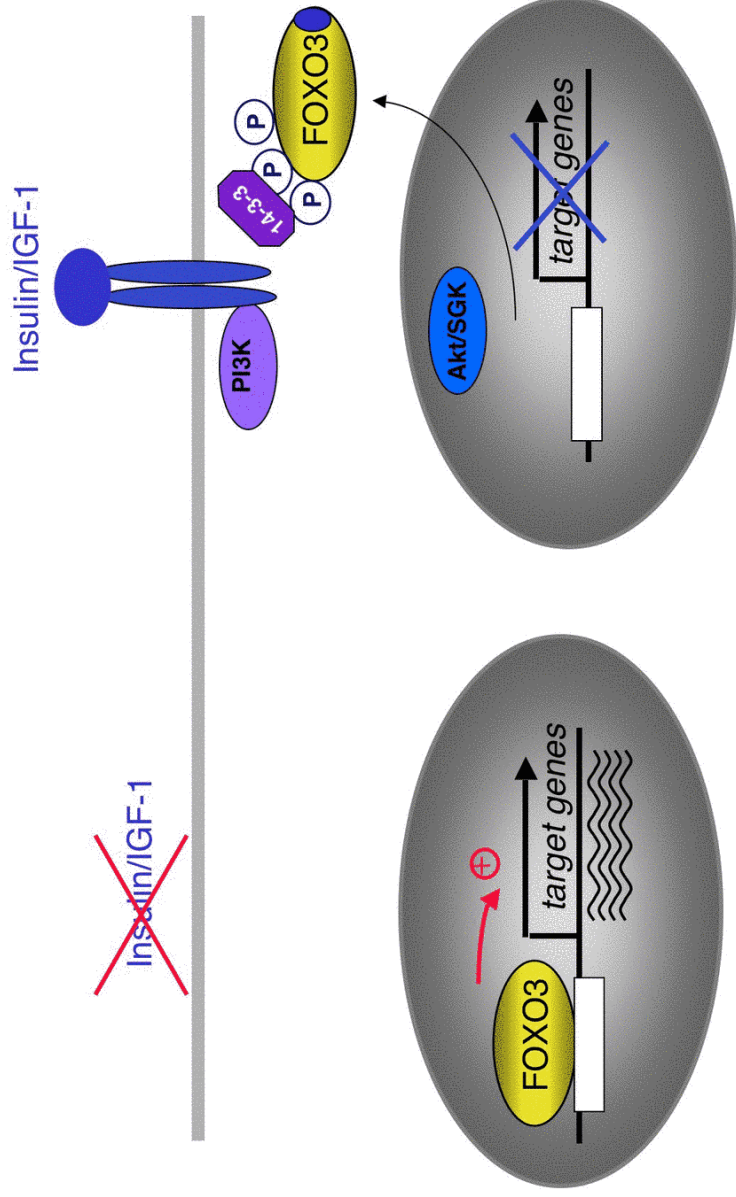
### Regulation of FOXO TF in mammalian cells



### Regulation of FOXO TF in mammalian cells



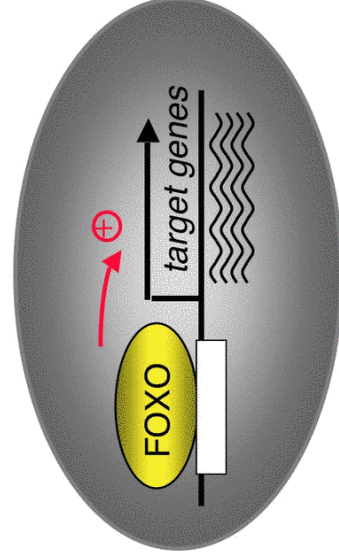
## Regulation of FOXO TF in mammalian cells



## Target genes and Function of FOXO in mammals

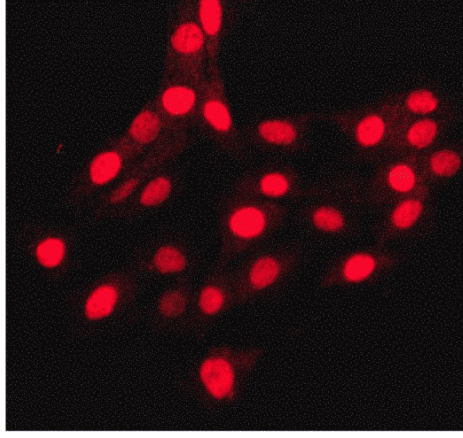
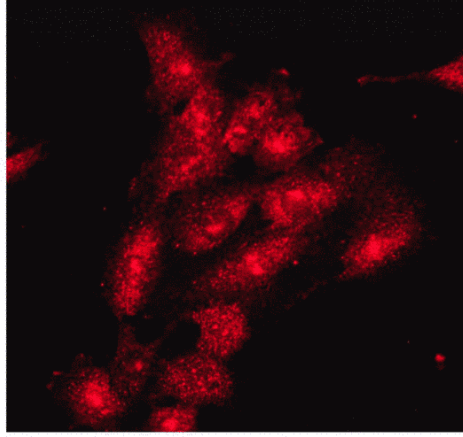
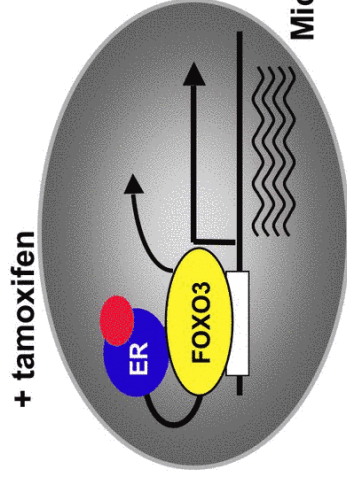
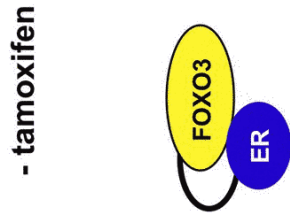
**Genome wide screen for FOXO target genes in mammals**  
**Micro-arrays**

~~Insulin/IGF-1~~

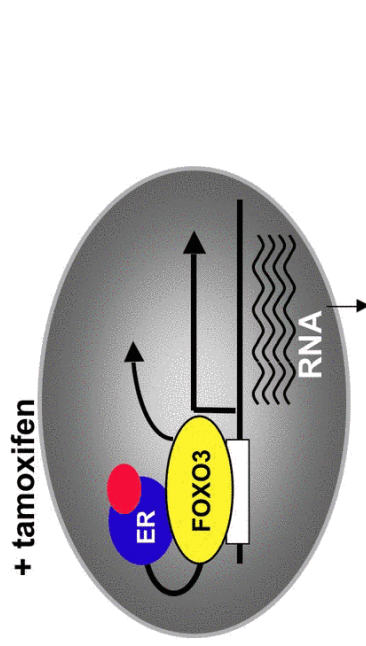
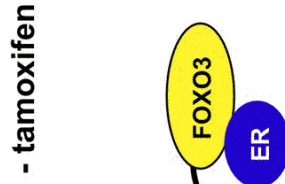


**Longevity**

# Inducible form of FOXO3



# FOXO target genes



-Millennium arrays (12000)  
-Affymetrix arrays (8000)

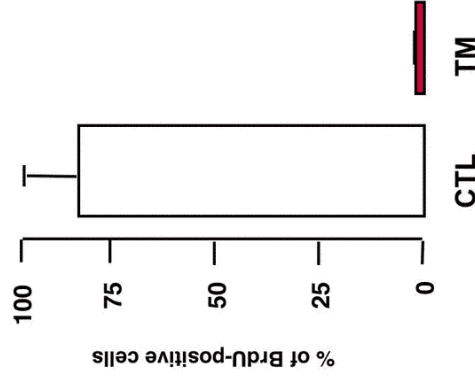
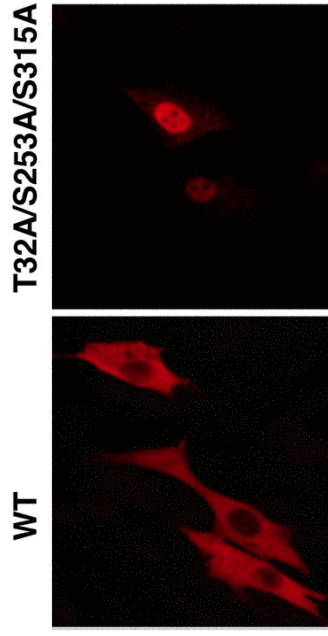
**CELL CYCLE ARREST**  
Ext-1  
p27KIP1  
Cyclin G2

**REPAIR OF DAMAGED DNA**  
GADD45  
PA26

**CELL DEATH**  
Fas ligand  
bNIP3  
BIM  
Legumain

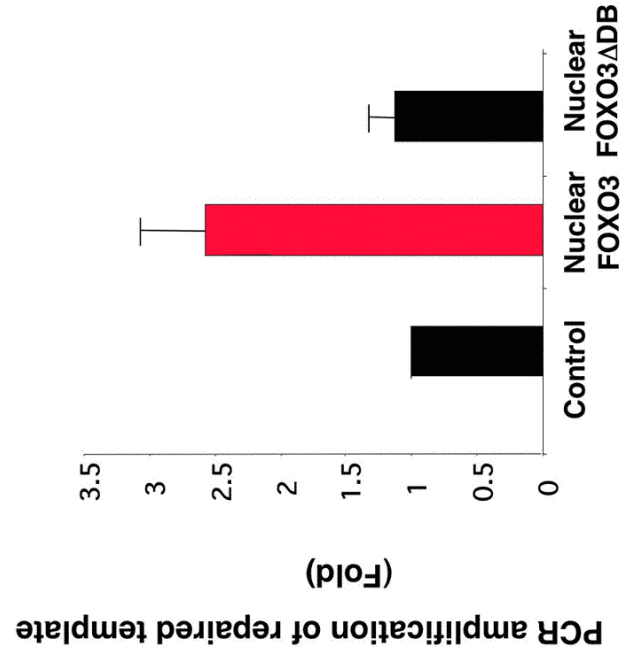
**DETOXIFICATION FROM ROS**  
Selenoprotein P  
MnSOD

## FOXO TFs induce cell cycle arrest in G1

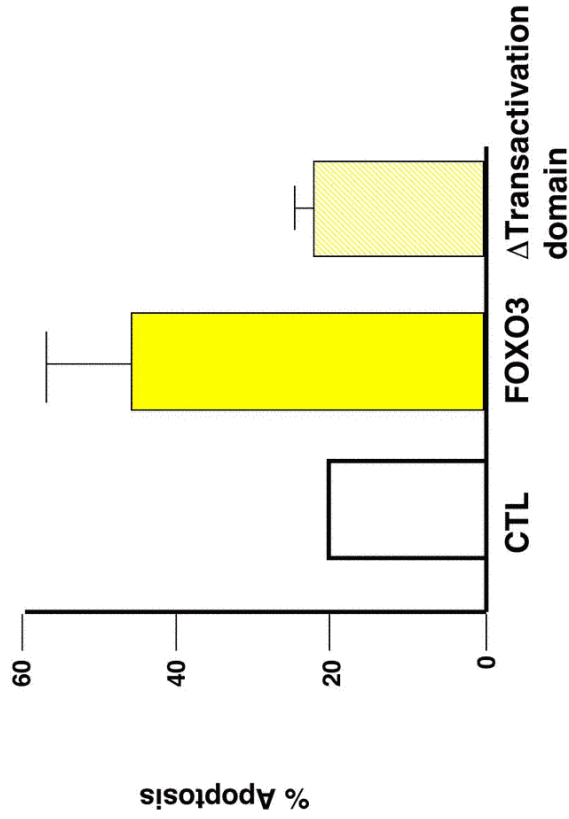
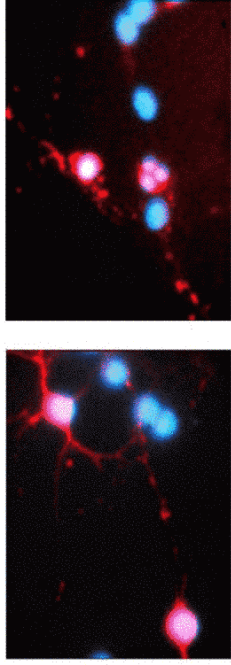


## FOXO3 induces DNA Repair

### Real time PCR



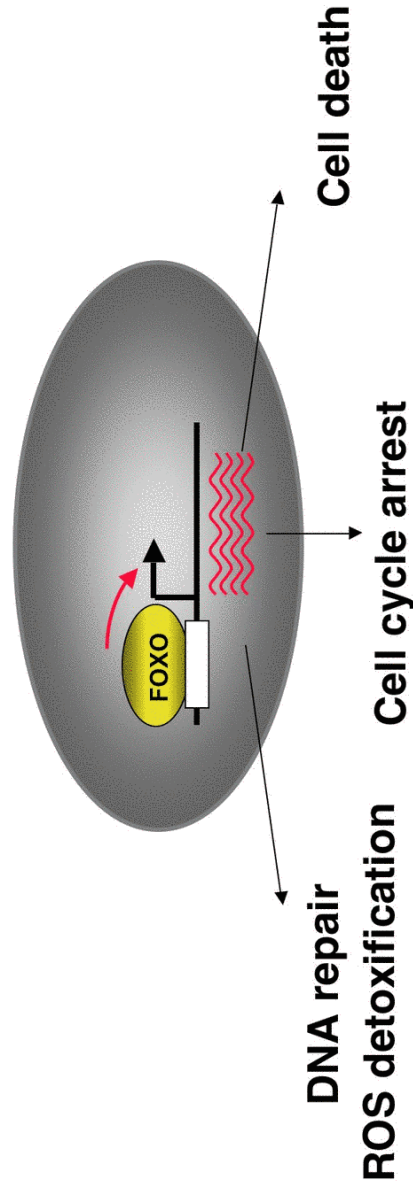
## FOXO3 induces neuronal death



## Cellular responses triggered by FOXO

**Extracellular conditions**

?



**Stress resistance**

## Environmental control of lifespan

**Restriction in caloric intake (40%)**

**Chronic low levels of oxidative stress**

**Resveratrol (red wine)**



**Increase longevity**

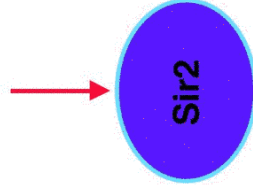
*worms spiders flies mouse rat baboon*

## Sir2 and organismal longevity

**Caloric restriction**

**Oxidative stress (chronic low levels)**

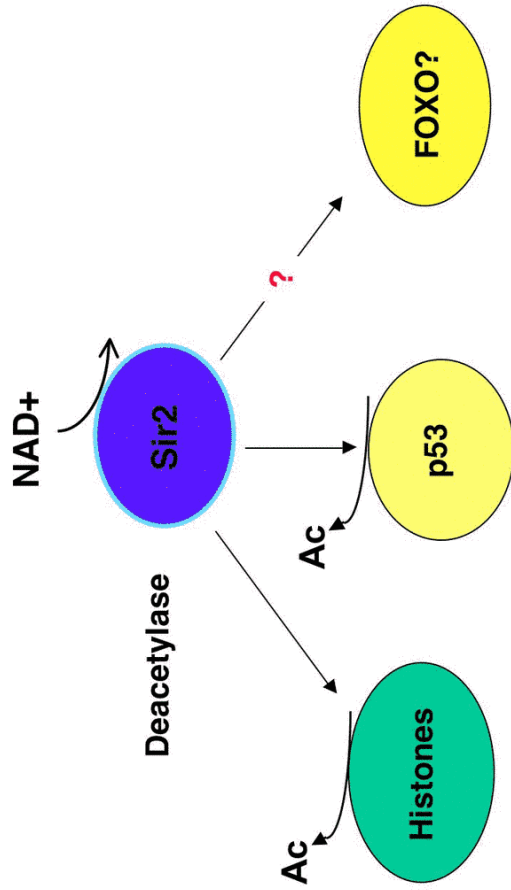
**Resveratrol (red wine)**



**Longevity**  
*Yeast, C. elegans, Drosophila*

# Sir2 and organismal longevity

Environmental cues



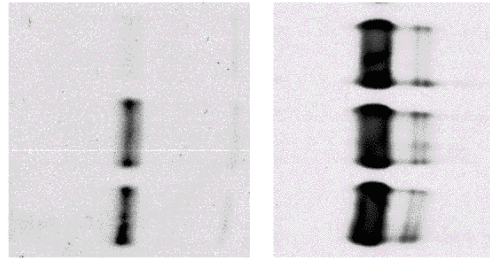
## Longevity

Yeast, *C. elegans*, *Drosophila*

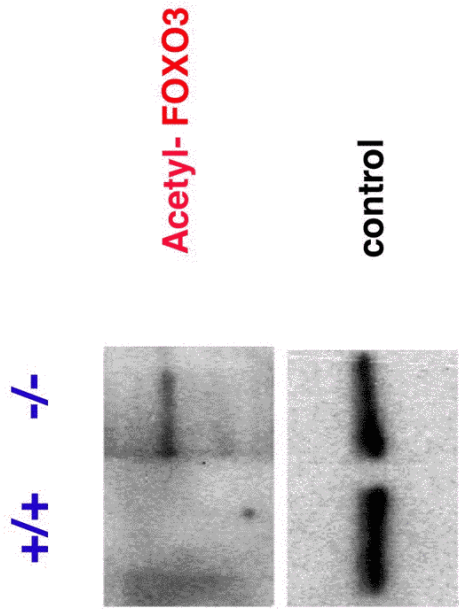
# Sir2 directly deacetylates FOXO3

Sir2

-  
- NAD+

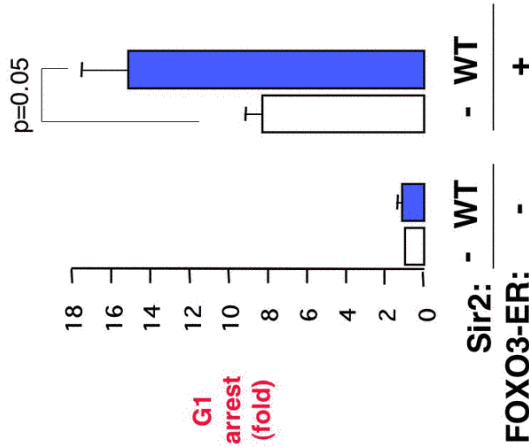


## Increased acetylation of FOXO3 in Sir2 $-/-$ cells

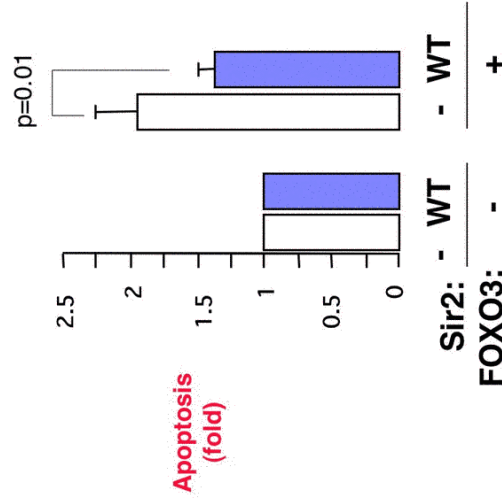


## Sir2 affects FOXO3-dependent cellular responses

**Stress resistance**



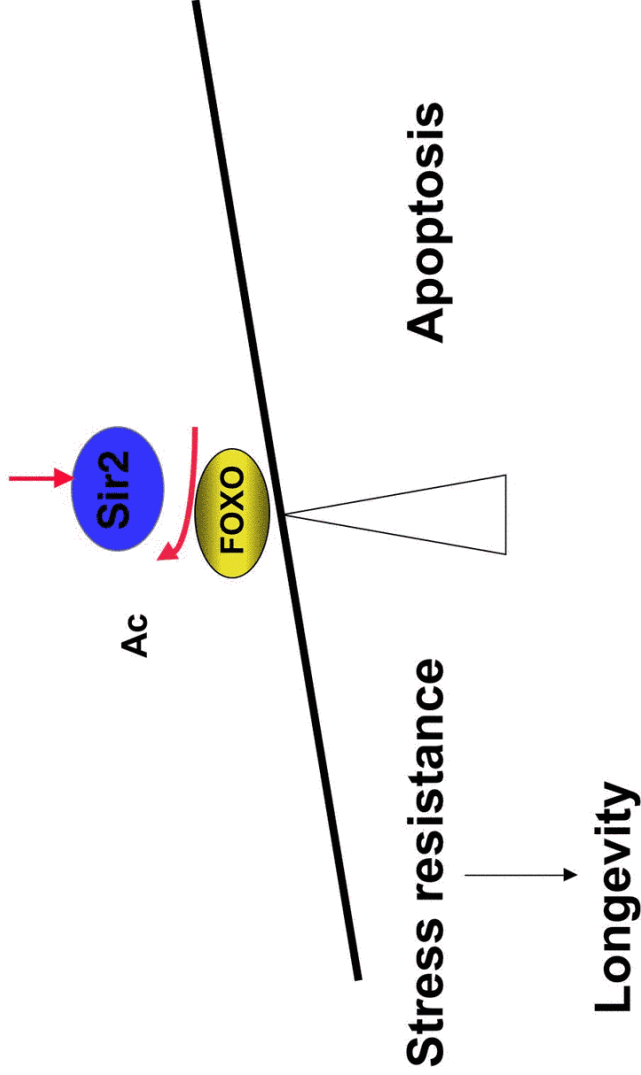
**Apoptosis**





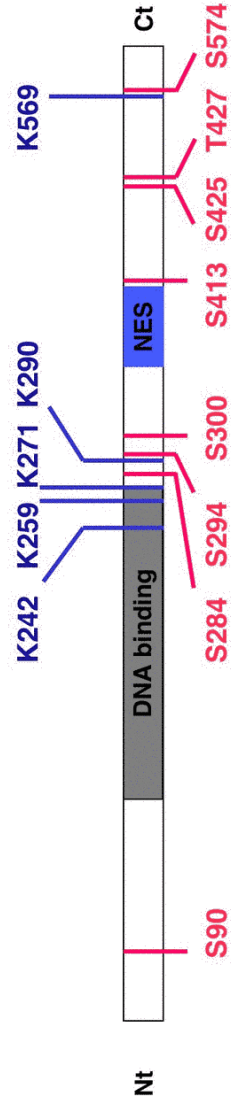
## Regulation of FOXO by Sir2

Extracellular conditions

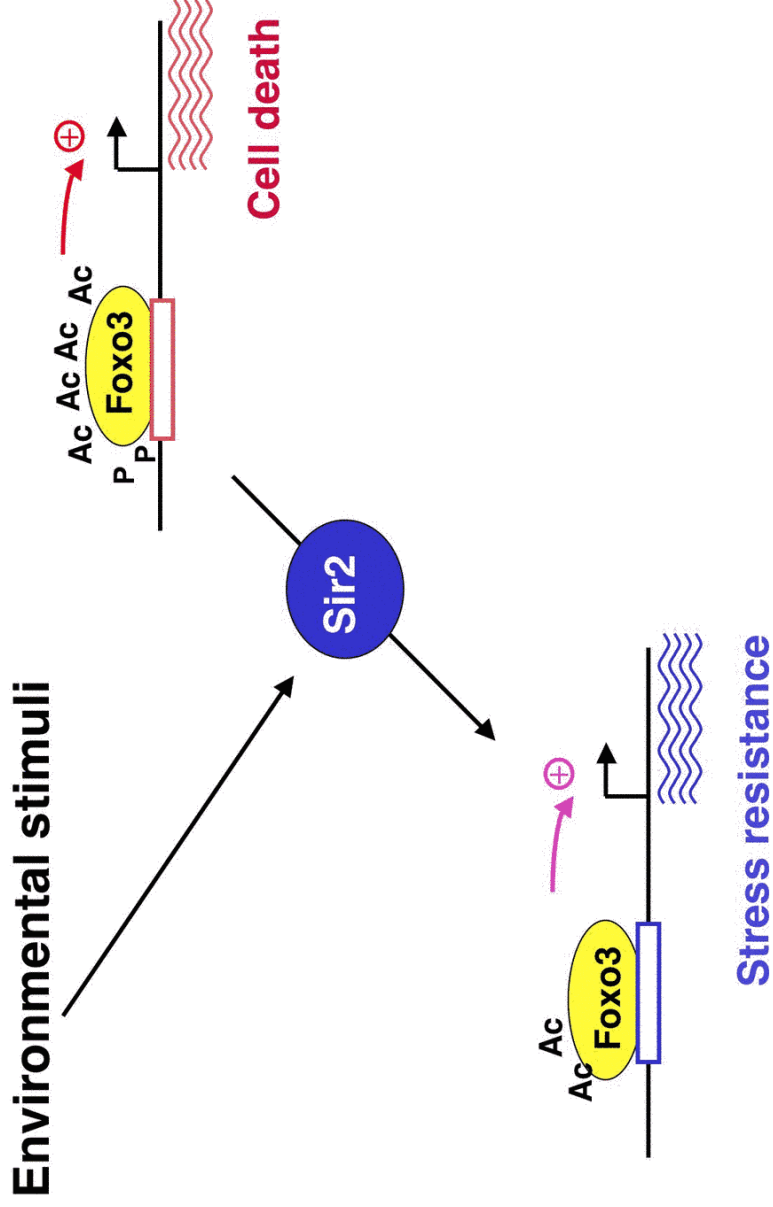


## Molecular signature of FOXO3

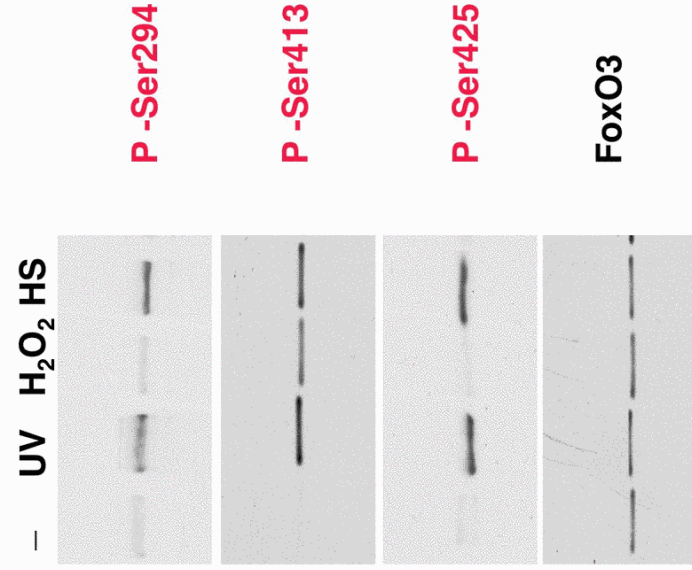
### Tandem mass spectrometry



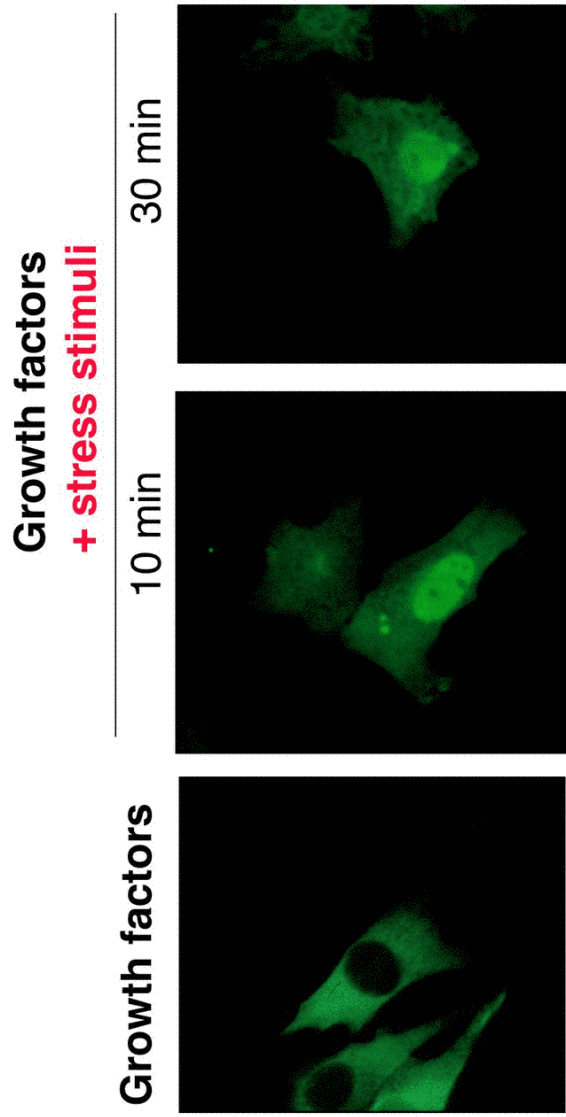
## Working model



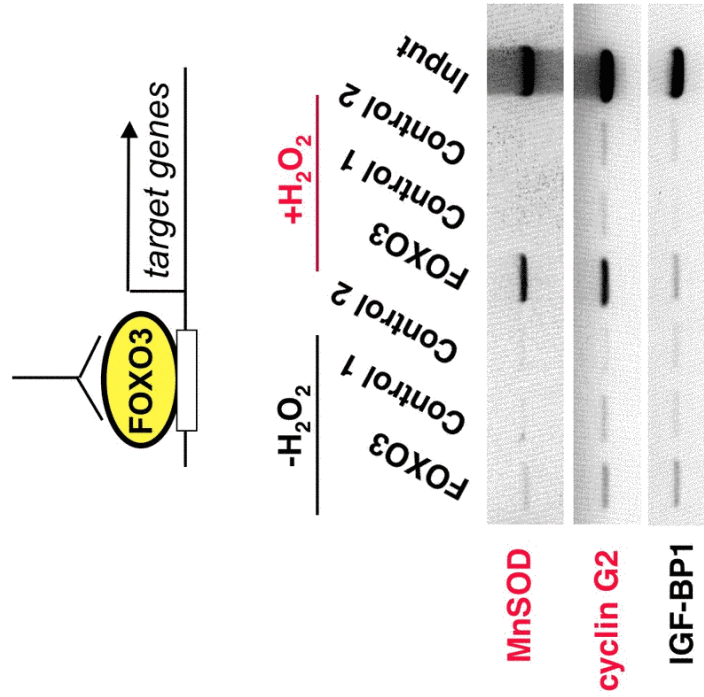
## Stress-induced phosphorylation of FOXO3



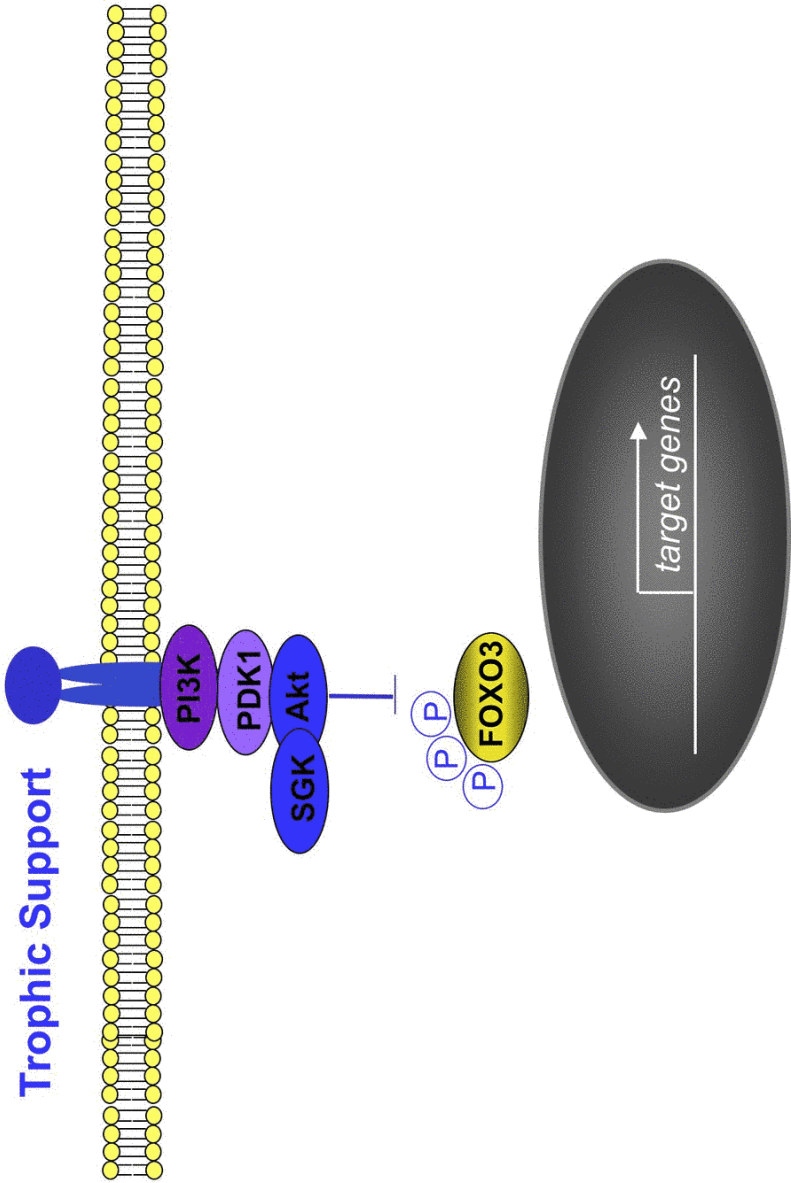
## Localization of FOXO3 in response to stress



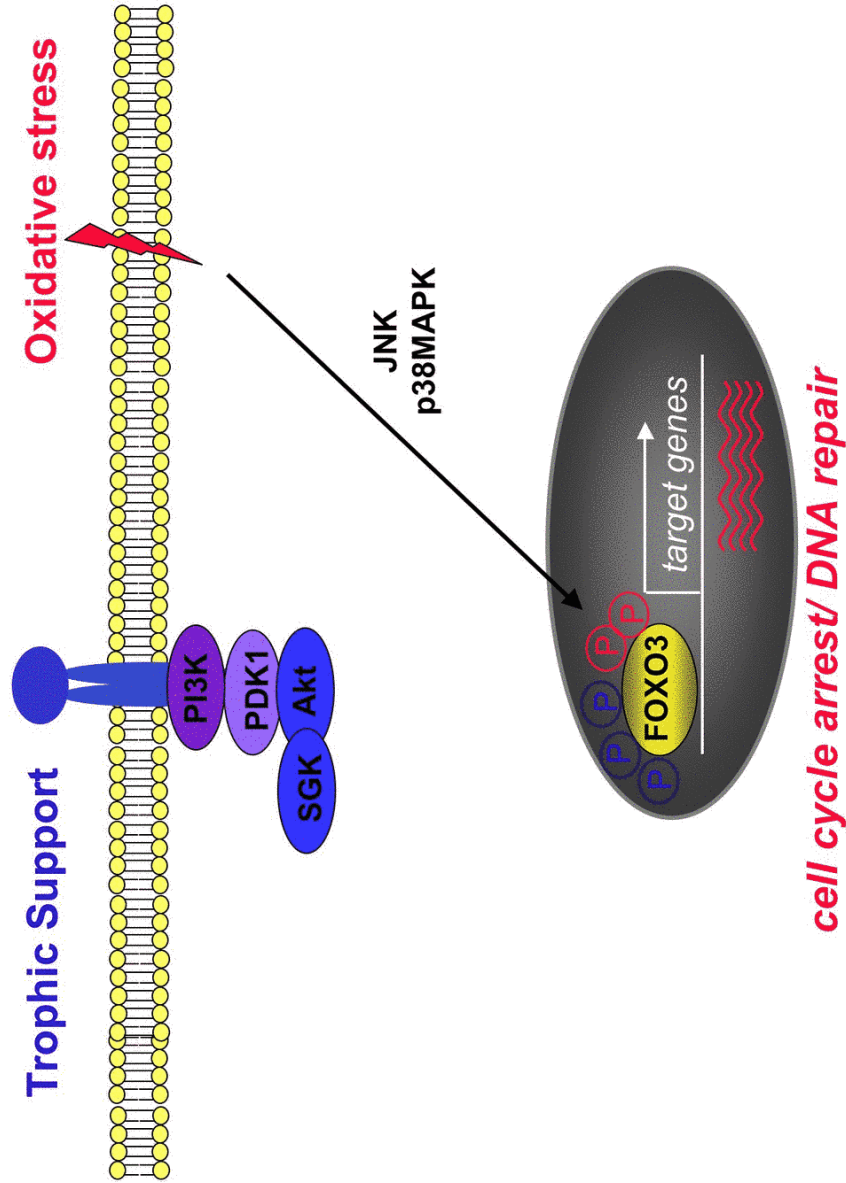
## Recruitment of FOXO3 to stress promoters



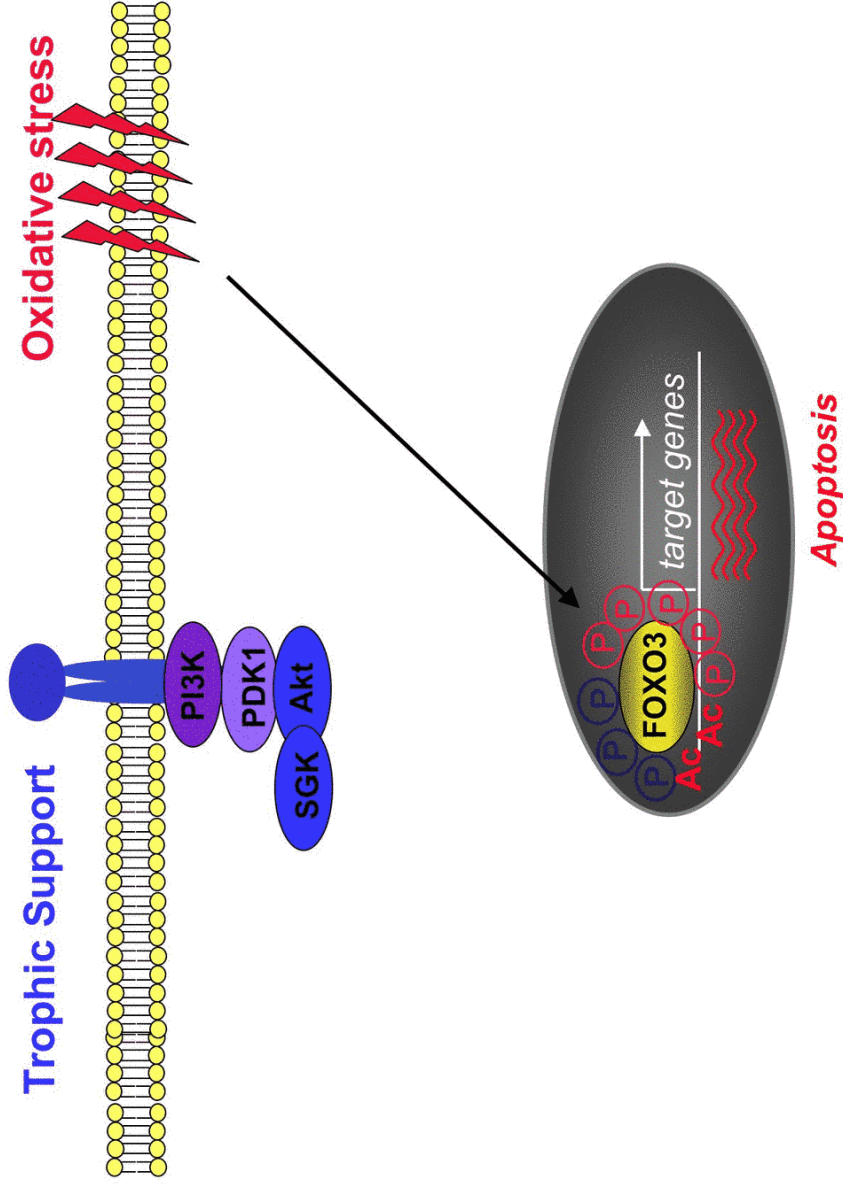
### Role of FOXO3 in the response to stress



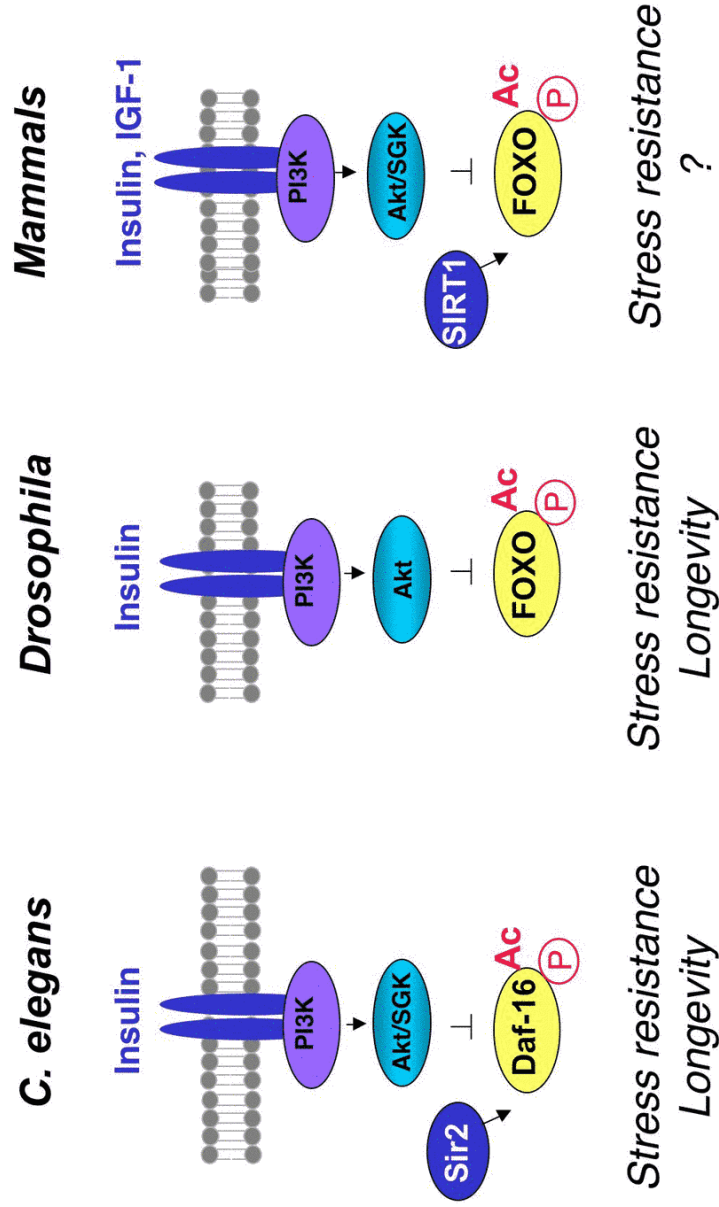
### Role of FOXO3 in the response to stress



## Role of FOXO3 in the response to stress

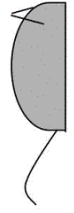


## Conservation of the pathways

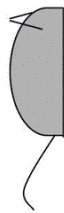


## Role of FOXO in longevity

FOXO inactive  
(knock-out, shRNA) → Accelerated aging  
Suppress longevity of CR?

A simple schematic of a protein structure, consisting of a grey oval with a tail extending from the bottom.

FOXO active  
(knock-in of active form) → Extended lifespan?

A simple schematic of a protein structure, consisting of a grey oval with a tail extending from the bottom.

Importance of the nervous system in  
regulating lifespan

CNS regions involved?

Longevity or aging factors?

## Regulation of organismal longevity



**Jeanne Calment, 122**

**Stanford University, Department of Genetics  
Brunet lab**

- Dan Calnan**
- Matt Carter**
- Eric Greer**
- Phil Oskoui**
- Ophelia Venturelli**

