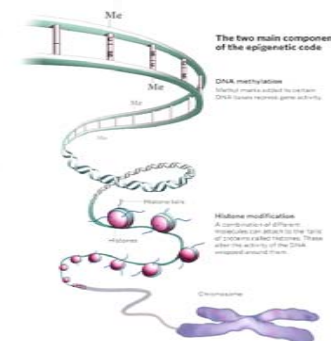
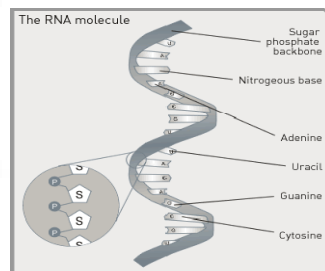
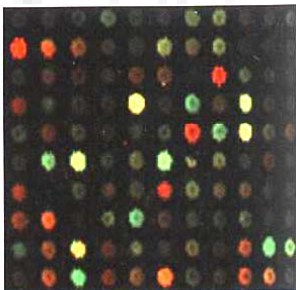


The role of Sirt1 in restricted energy intake

Dianne Ford
American Aging Association - 37th Annual Meeting
Trans-Atlantic Awareness and Collaboration
Symposium on Aging Research
31 May 2008

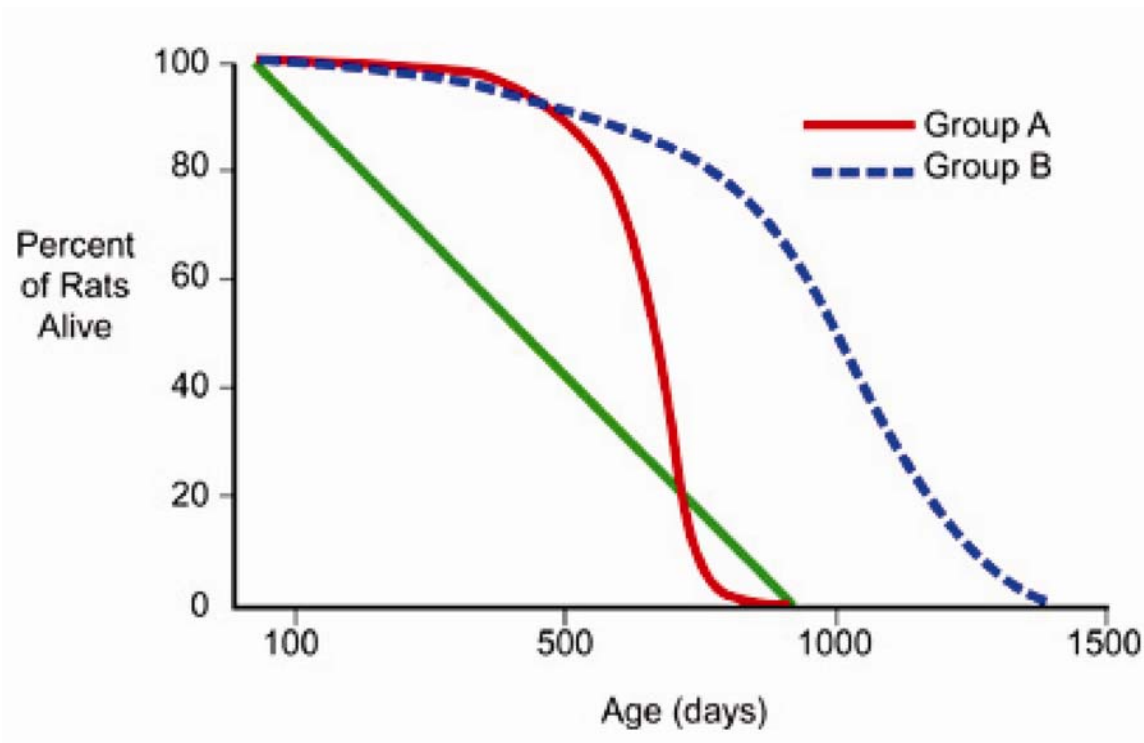


Dietary restriction (DR) increases lifespan in evolutionarily-distinct species

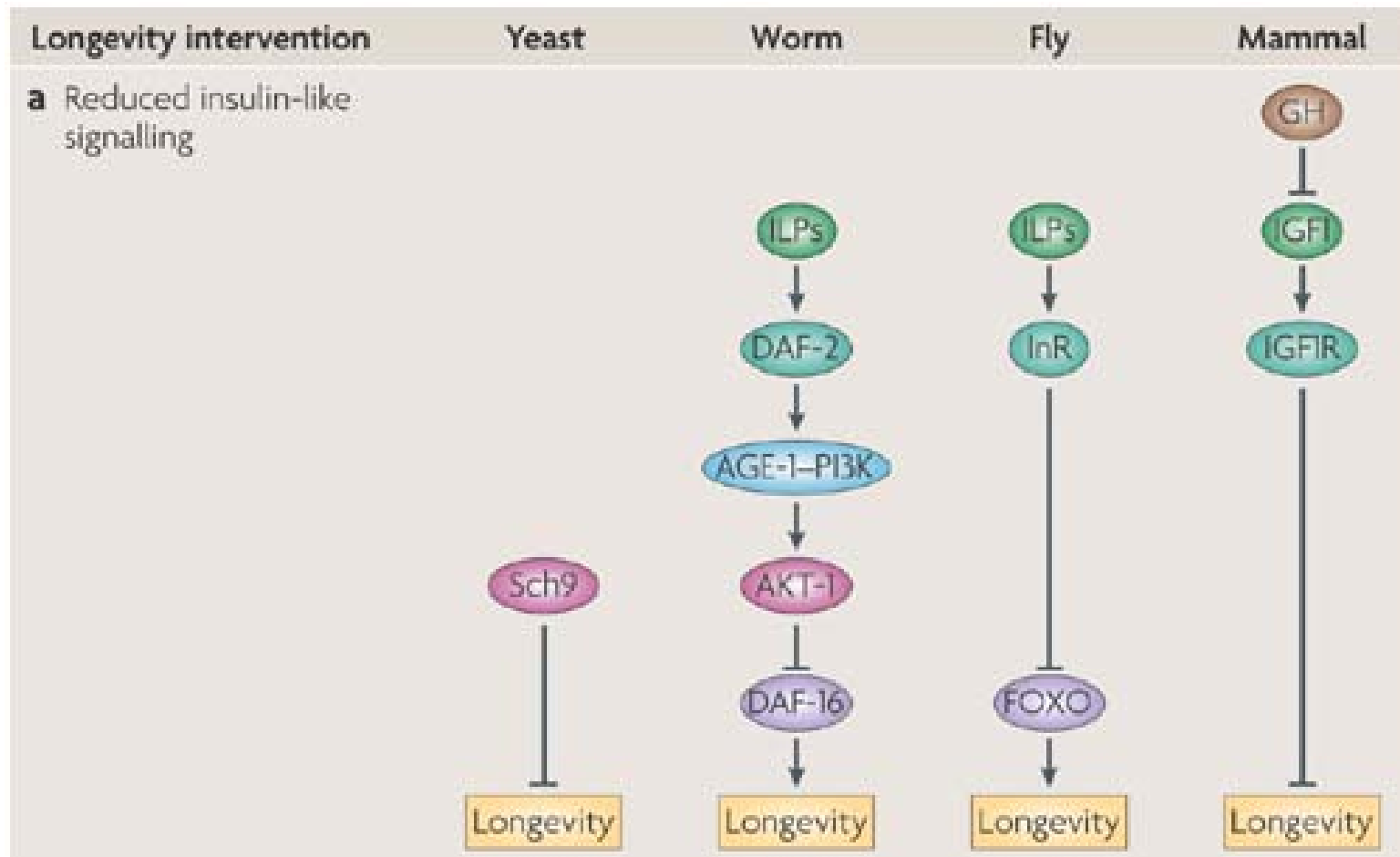
- *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Drosophila melanogaster*, rodents

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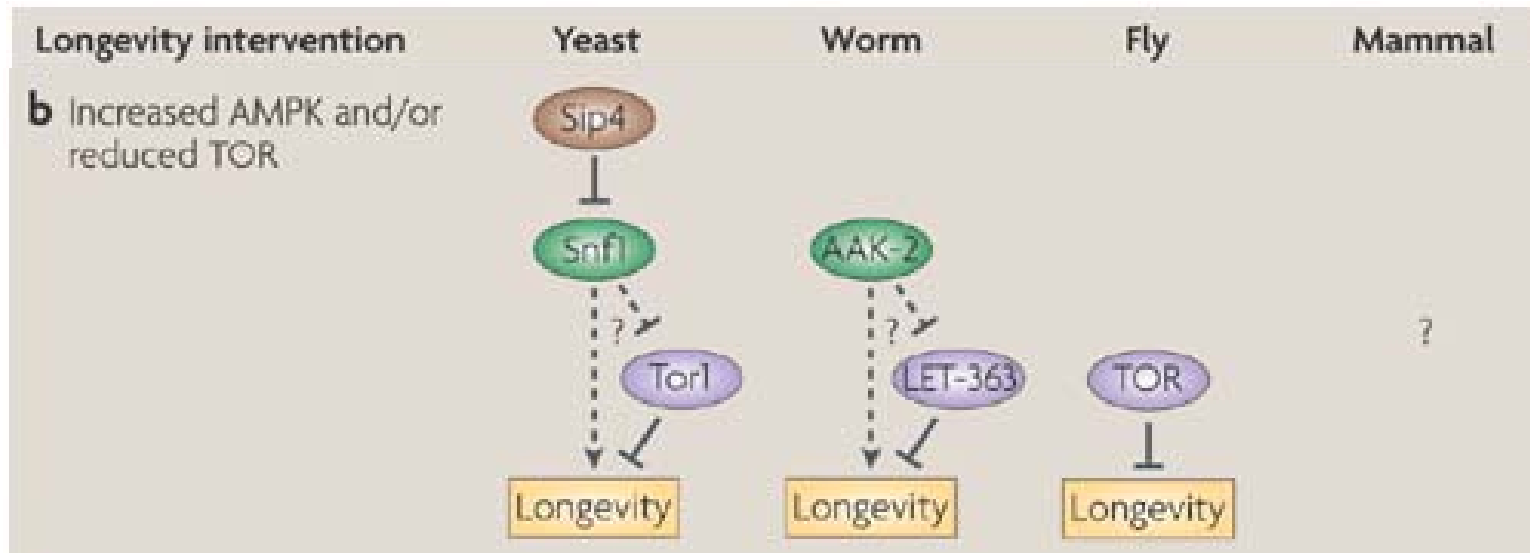
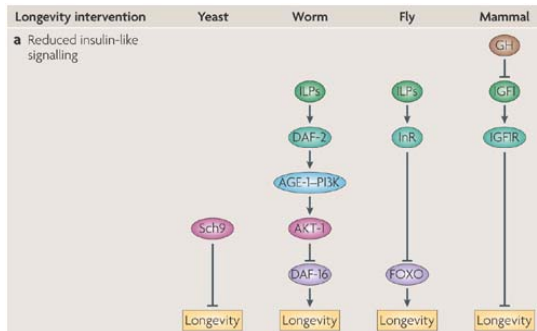


Conserved lifespan control pathways

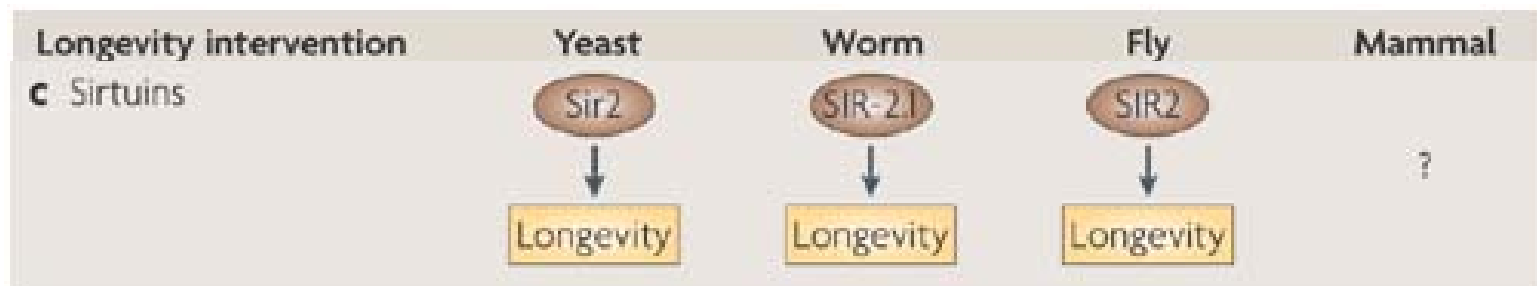
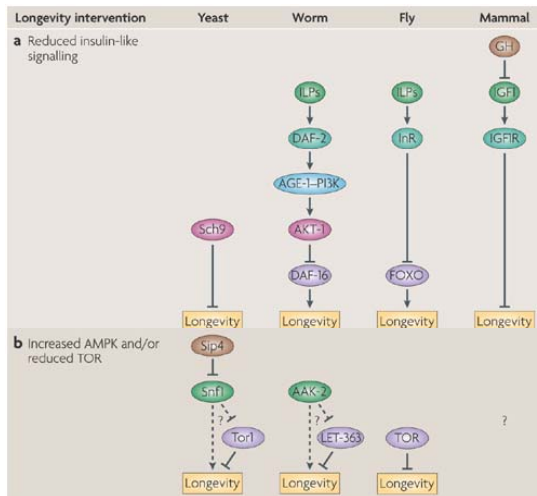


From: Bishop NA & Guarente L (2007) *Nature Reviews Genetics* 8: 835-844 

Conserved lifespan control pathways

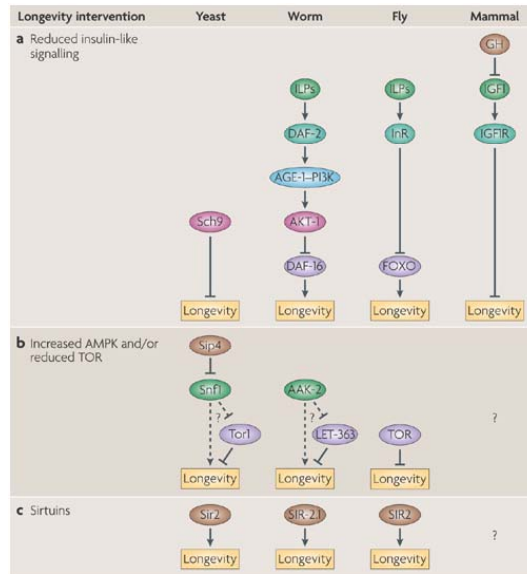


Conserved lifespan control pathways



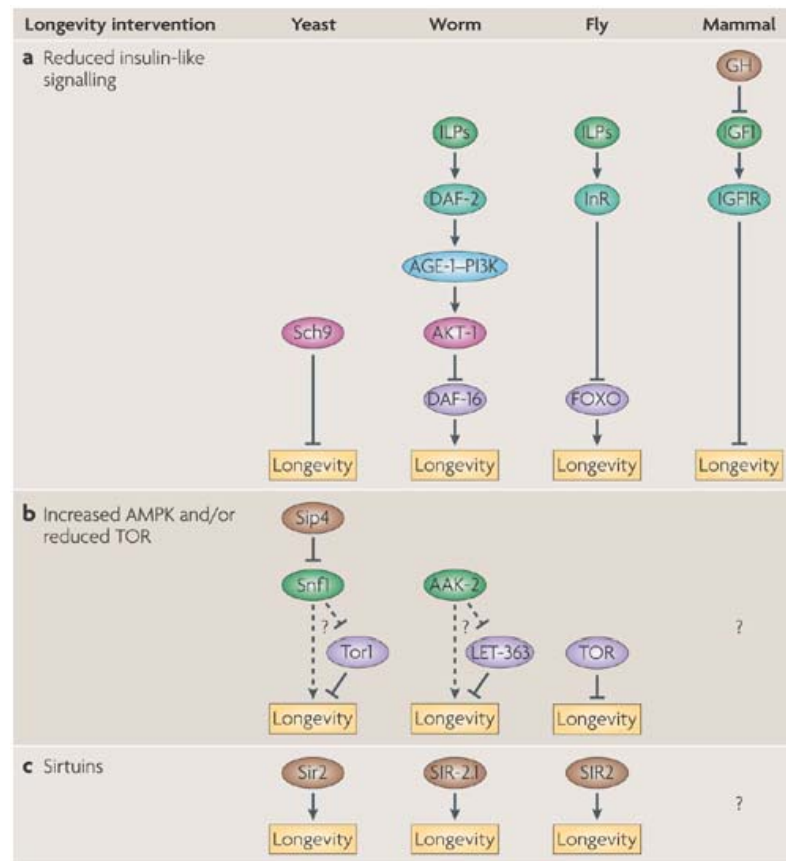
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Conserved lifespan control pathways



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Conserved lifespan control pathways



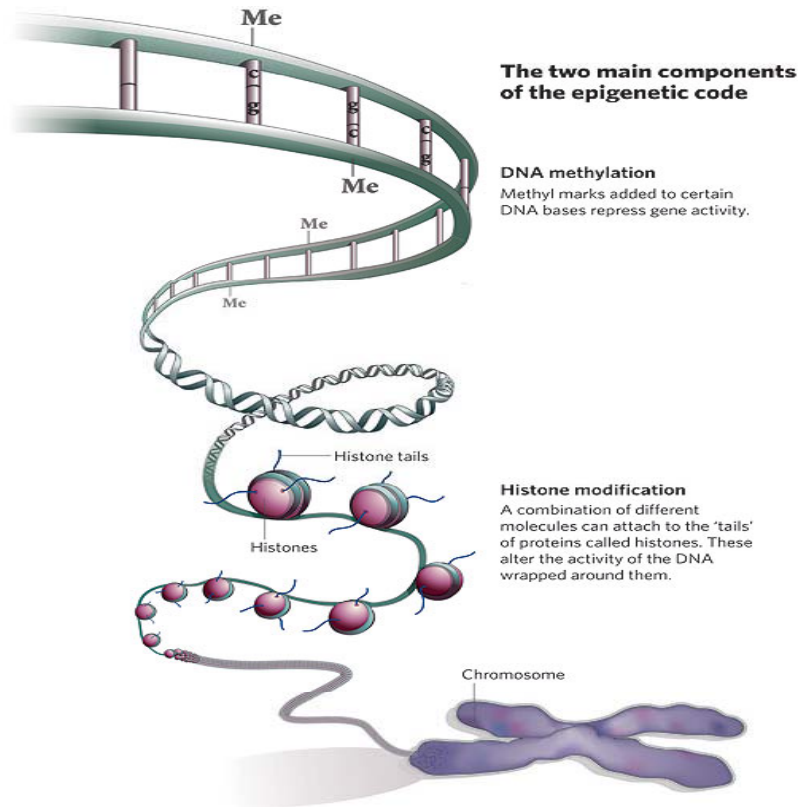
From: Bishop NA & Guarente L (2007) *Nature Reviews Genetics* 8: 835-844

Sirt1

- Mammalian Sirt1, or homologues (Sir2 in yeast), appears to be pivotal in the effect of DR on lifespan
- Multiple actions/targets and implicated in regulation of various aspects of DR responses
 - Substrates for deacetylation
 - PGC-1 α
 - P53
 - Mitochondrial uncoupling protein 2 gene (*UCP2*)
 - Pathways/responses
 - Glucose homeostasis
 - Insulin secretion
 - Fat metabolism
 - Stress resistance
 - Physical activity
- NAD-dependent histone deacetylase (Class III)

Hypothesis: Sirt1 affects lifespan/ageing through effects on DNA methylation, mediated via effects on histone acetylation.

Epigenetic modification of DNA



Methylated DNA is associated with deacetylated histones

Qui, J (2006) *Nature* 441: 143-145

Hypothesis: Sirt1 affects lifespan/ageing through effects on DNA methylation, mediated via effects on histone acetylation.

DNA methylation and ageing

- Ageing-associated changes in DNA methylation are observed.
 - Decrease in total deoxymethylcytosine levels
 - Regional hyper- and demethylation
- Aberrant DNA methylation can result in inappropriate gene expression or gene silencing.
- Changes in DNA methylation may be causal in the ageing process.

Resveratrol

- Polyphenol, red grapes
- Extends lifespan in simple organisms (*S. cerevisiae*, *Drosophila*, *C. elegans*) in Sir2-dependent manner
- *In vitro* Sirt1 activator (?)
- Improved health and survival of mice on high fat diet and induced changes associated with longer lifespan
 - Increased insulin sensitivity
 - Reduced IGF-1 levels
 - Increased AMP-activated protein kinase activity
 - Increased PPAR-activated receptor- γ coactivator 1 α (PGC-1 α) deacetylation (activity)
 - Data consistent with Sirt1-mediated deacetylation

Aim

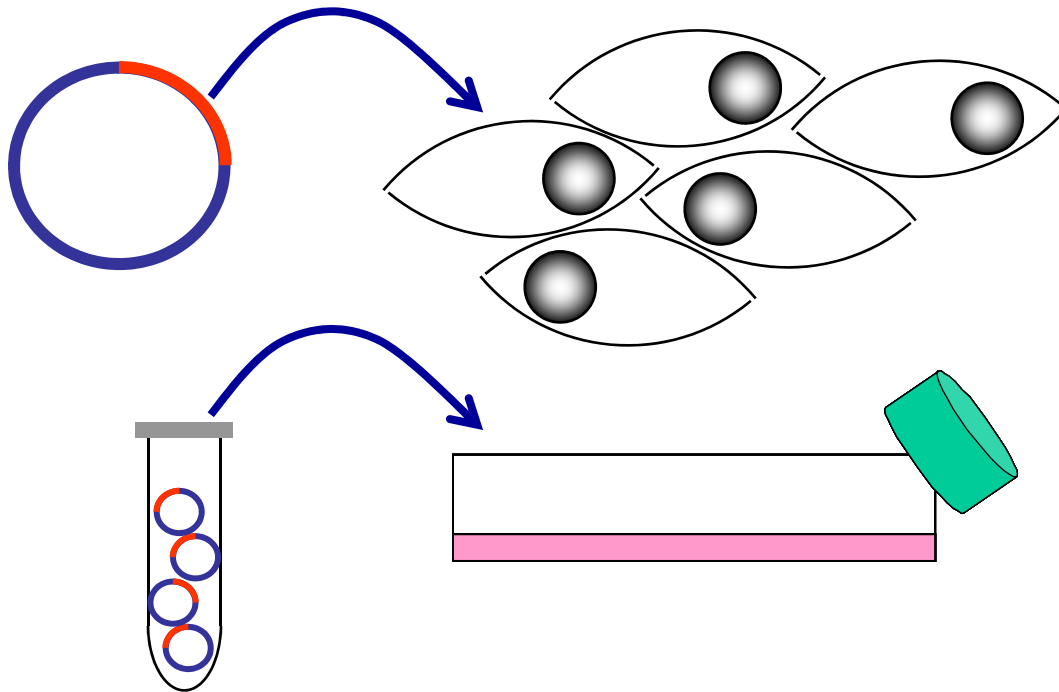
- Establish proof of concept that increased Sirt1 expression, influenced by resveratrol, can affect DNA methylation

Approach

- Express high levels of human Sirt1 in cultured human cells (Caco-2) from a transgene, in presence and absence of resveratrol, and measure DNA methylation compared with control cells (vector only).

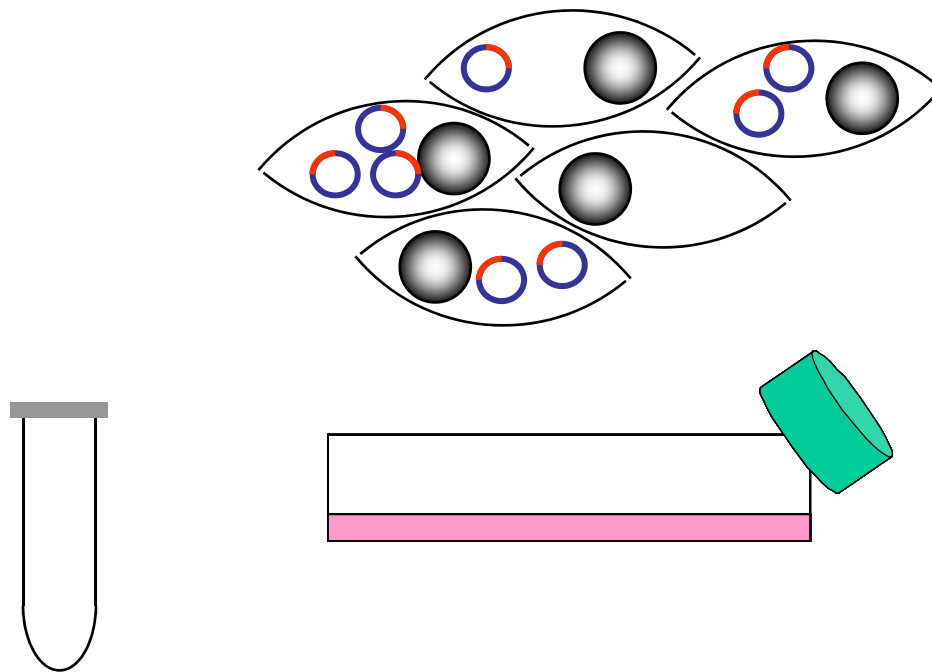
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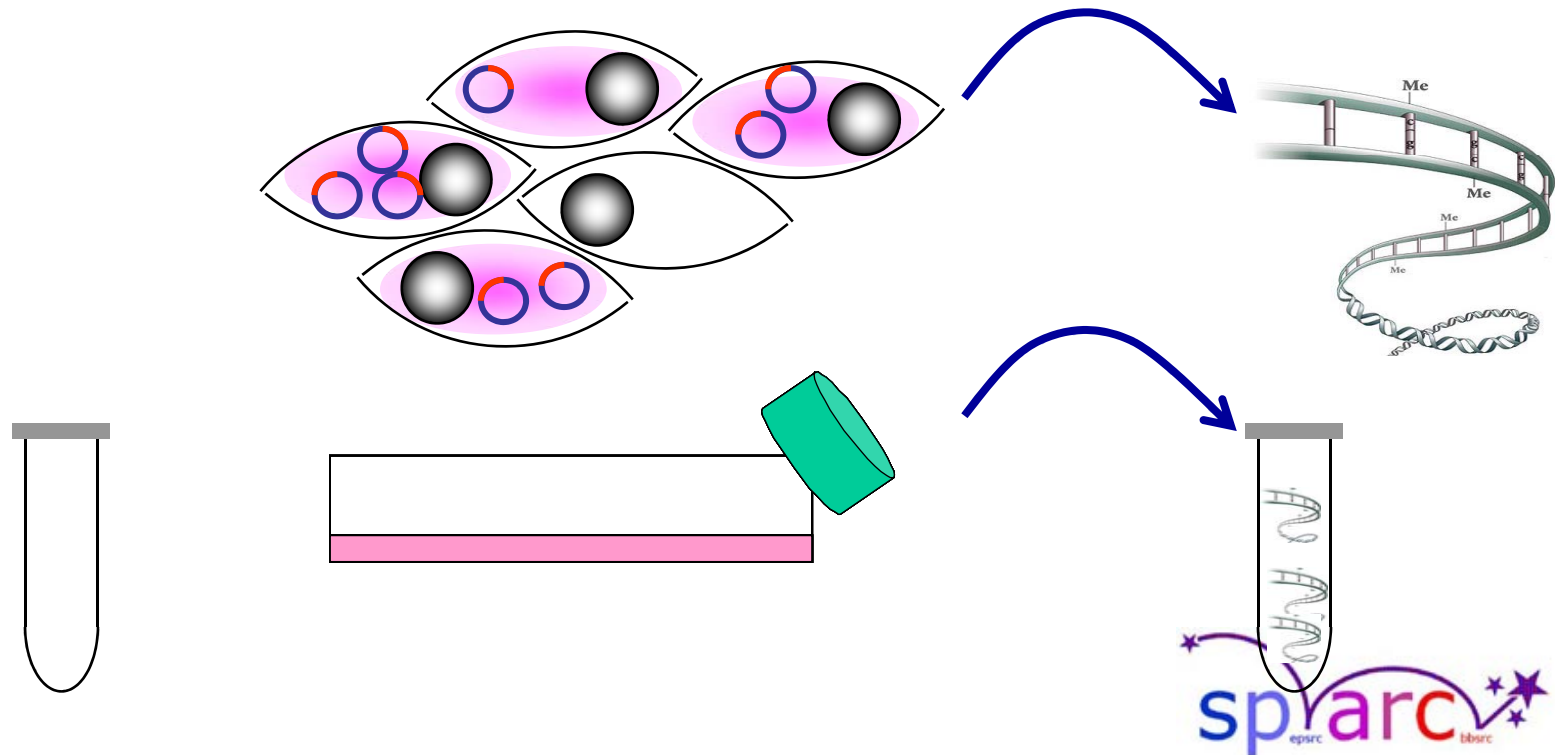
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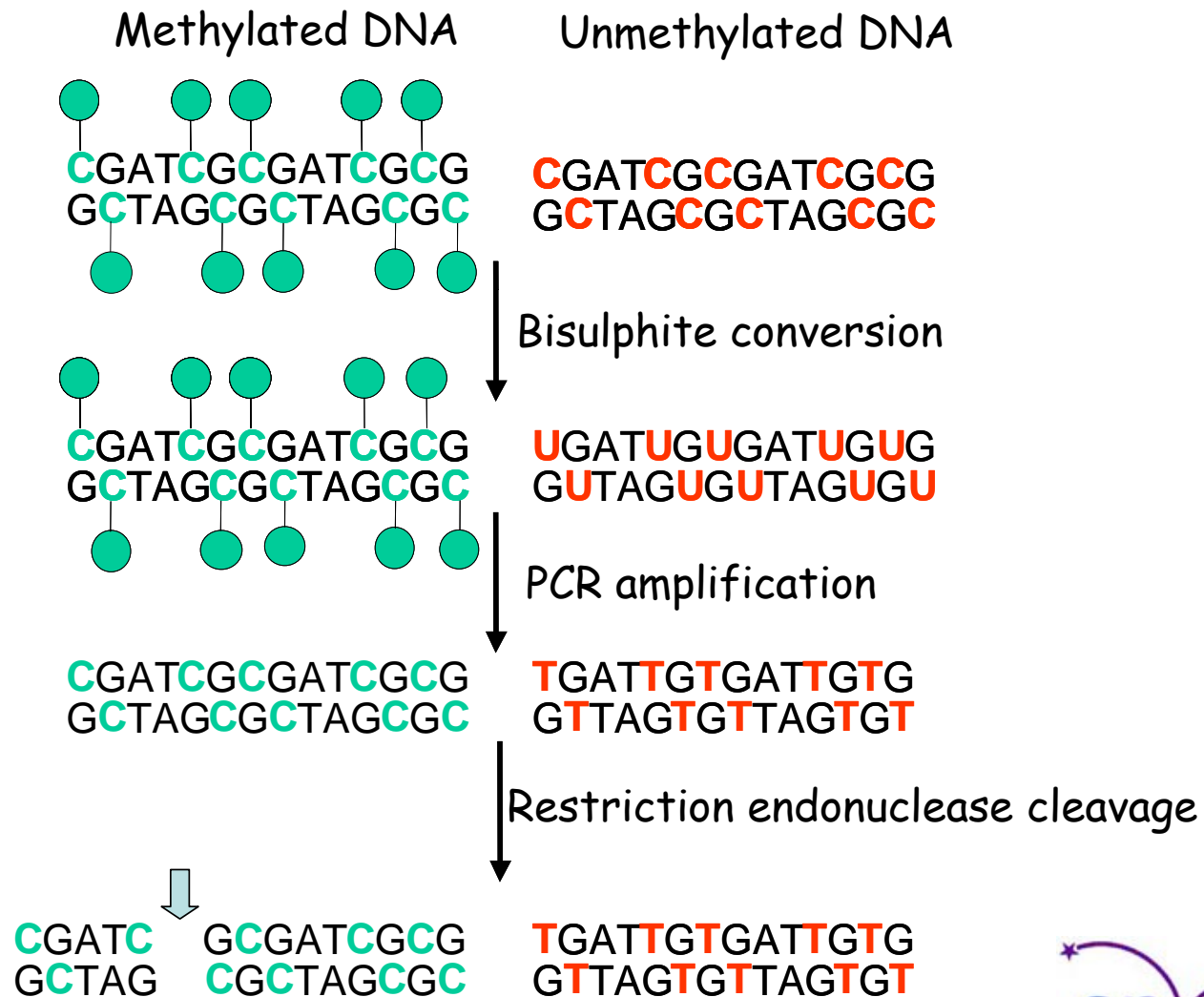


Approach

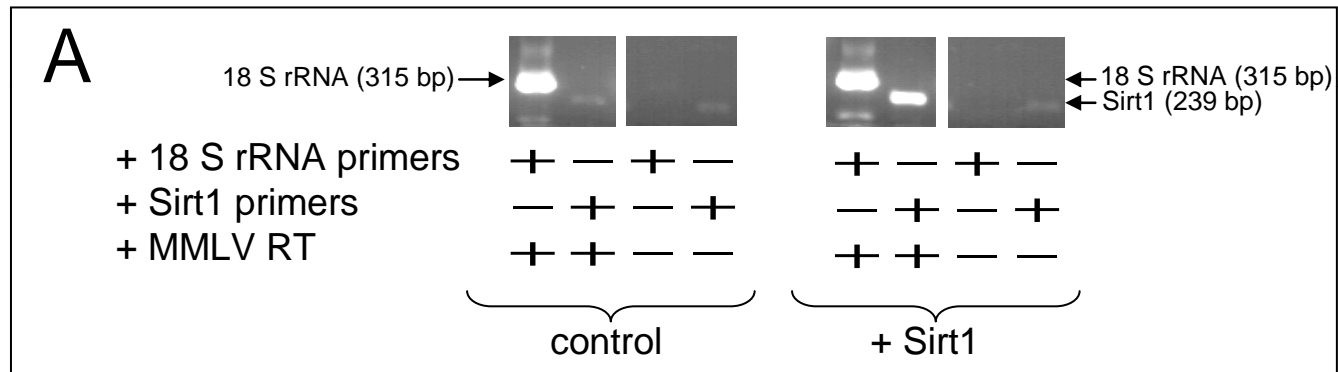
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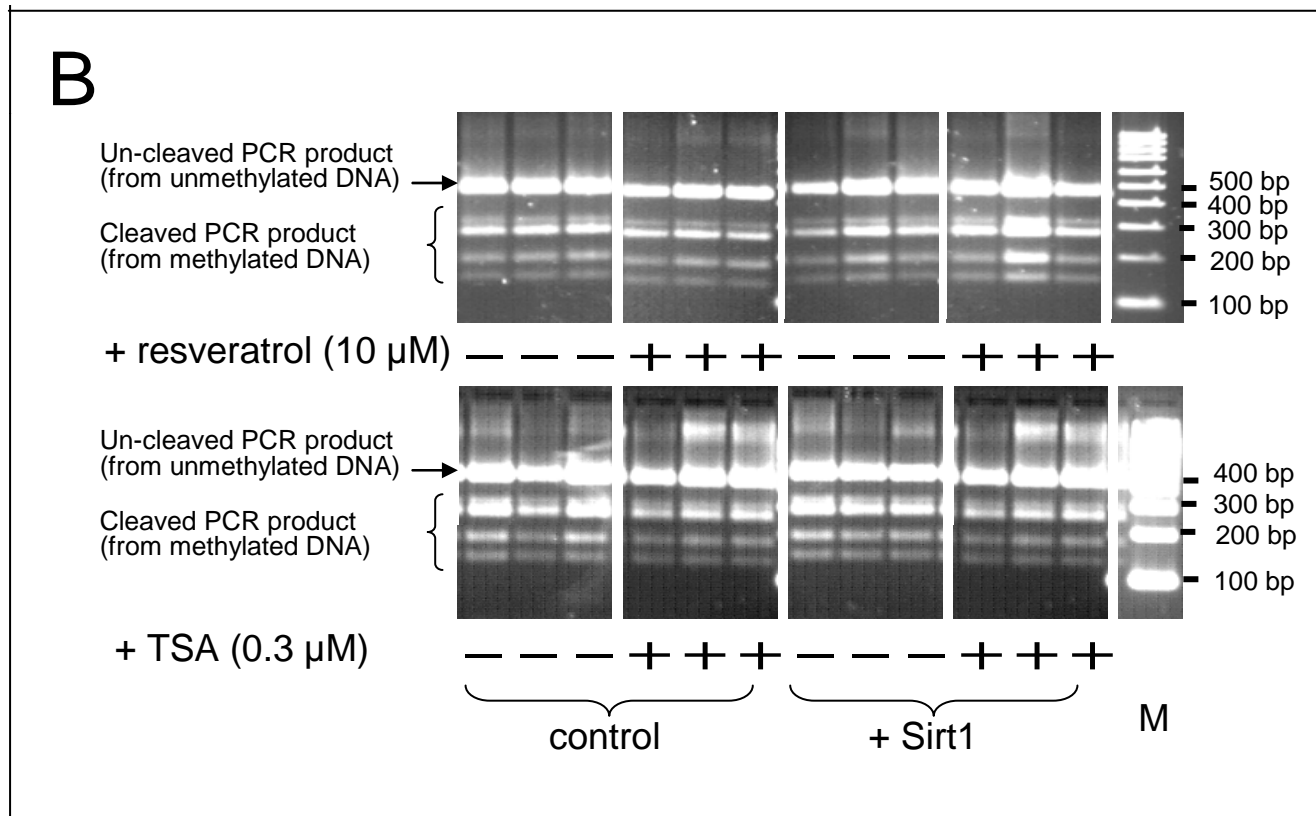
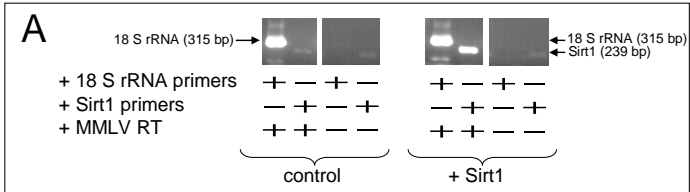
Measurement of DNA methylation - COBRA assay



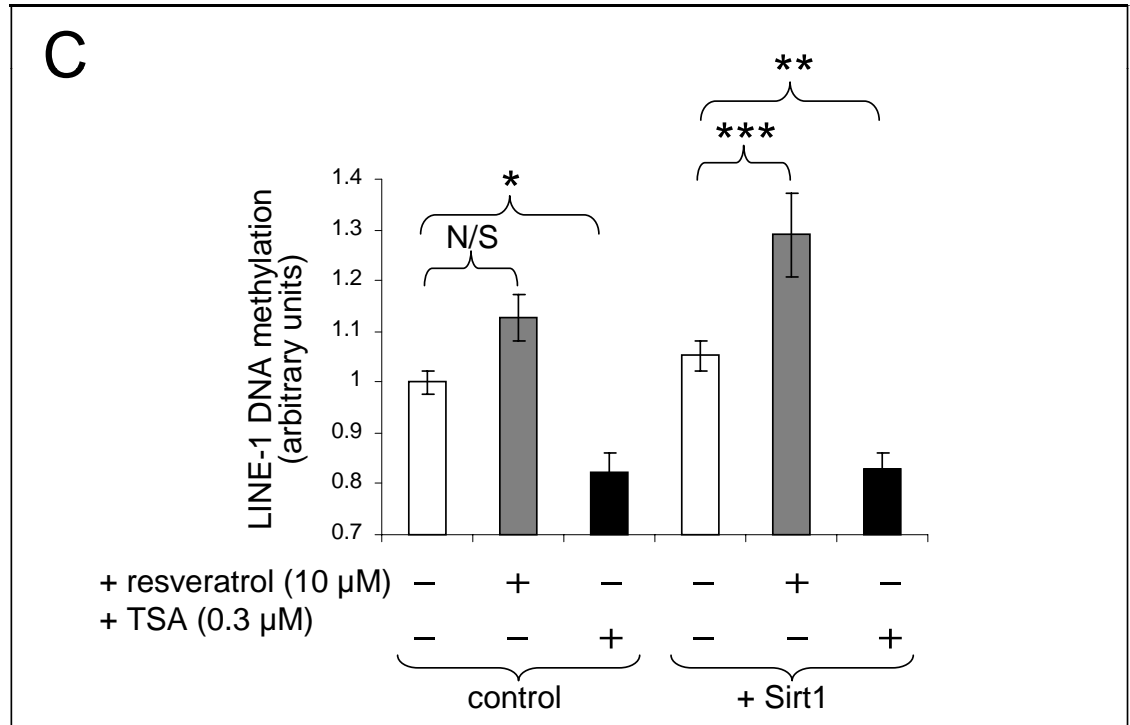
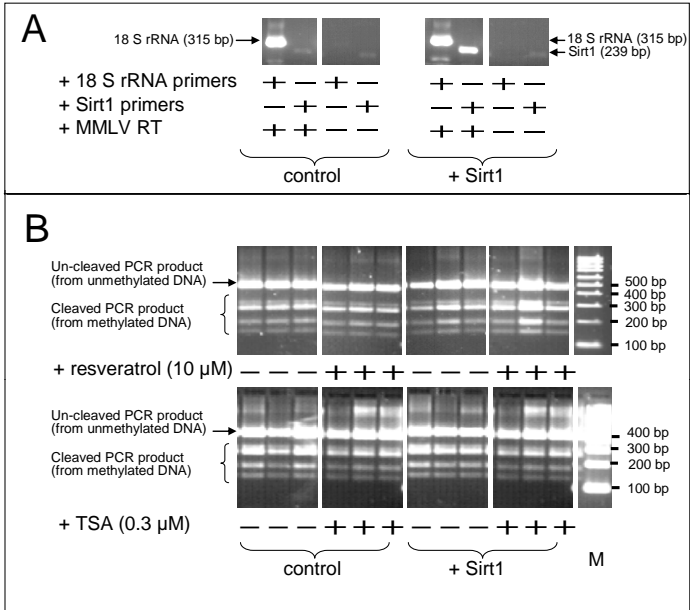
Results



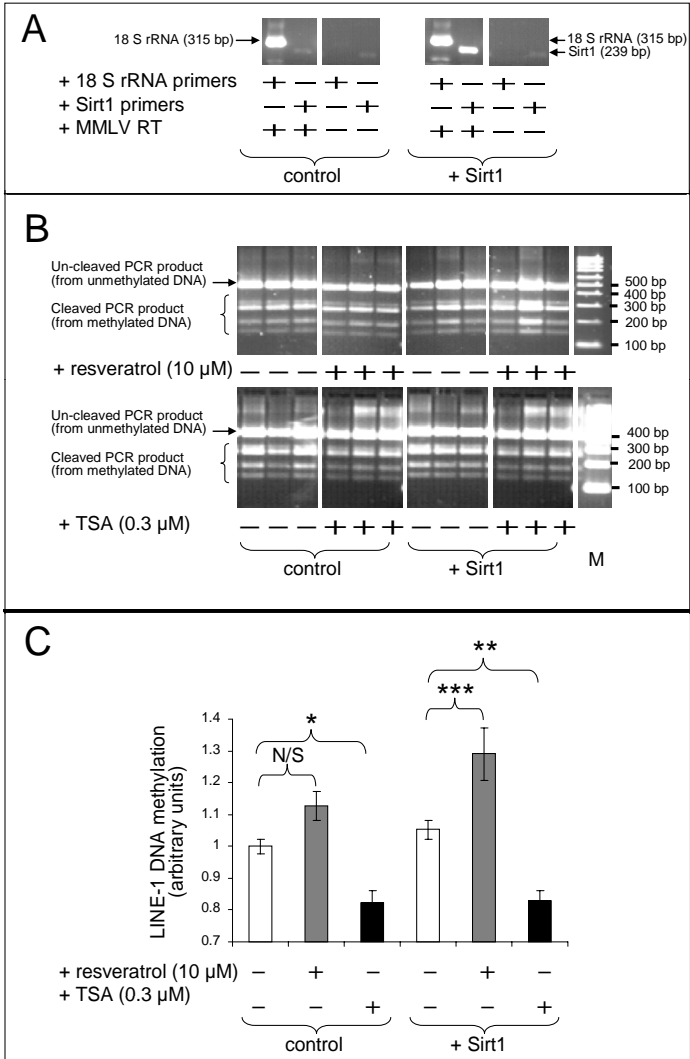
Results



Results



Results



- TSA (Class I/II HDAC inhibitor) reduced methylation of LINE-1 elements by 18% in Caco-2 cells.
- Increased expression of Sirt1 in combination with resveratrol increased methylation of LINE-1 elements by 30% in Caco-2 cells.

Conclusions

- TSA reduced methylation of LINE-1 elements in Caco-2 cells.
 - Adds to limited published evidence that DNA methylation can be influenced by changes in histone acetylation
- Increased expression of Sirt1 in combination with resveratrol increased methylation of LINE-1 elements.
 - Consistent with action through a common pathway
 - Sirt1 activation by resveratrol
 - Maintenance of DNA methylation patterns potentially mediates some effects of DR/resveratrol to delay aging/increase lifespan.
- Observations provide impetus for future studies to investigate effects of Sirt1/DR and resveratrol in cell lines and rodents on epigenetic modification at specific loci
 - May reveal potential genomic targets at which have a direct impact on the aging process
 - Opportunities for Trans-Atlantic collaboration?



Acknowledgements

- Luisa Wakeling
- John Mathers

