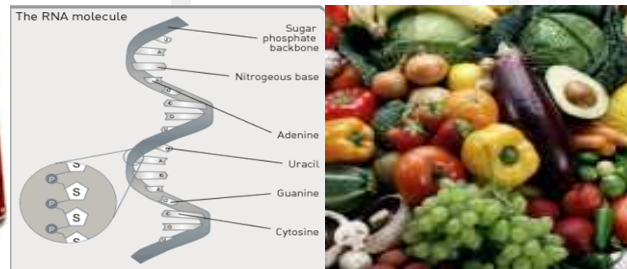


The molecular basis of the effects of calorie restriction on ageing

Dianne Ford
SPARC workshop - 11 January 2007



PI Background

- BSc Biochemistry
- PhD Biochemistry (molecular enzymology)
- Postdoctoral research on xenobiotic metabolism then on nutrient (peptide and amino acid) transport
- Current research focus "molecular nutrition"
 - Zinc transport/absorption and zinc-regulated gene expression
 - Isoflavone metabolism (contribution of genetic factors to inter-individual variability)
 - Nutritional epigenomics
 - Influences of nutrition on non-sequence-related modification of DNA
 - Collaboration with Professor John Mathers, Newcastle University

Nutrition and ageing



- Importance of nutrition for health well-established
- Great potential to modulate the ageing process through nutritional strategies, or therapies that mimic the effects of effective nutritional strategies.
 - Requires an understanding of effects of nutrition on ageing at the molecular level

Background to project - calorie restriction and ageing

- Well established (based on research going back several decades) that calorie restriction (approximately 60% normal intake in rodents) increases lifespan and/or reduces incidence of ageing-related disease in model organisms including yeast, *C. elegans*, *Drosophila*, mice.
- Applies in humans?



Calorie Restriction

Fewer Calories. More Life.

The Calorie Restriction Society

for people trying to live longer by eating fewer calories

Search:

Home

[About the CR Society](#)

[CR Guide](#)

[Society Services](#)

[Membership](#)

[Video Clips](#)

[Frequently Asked Questions](#)

[Contact Us](#)

Research that will change your life!

Since growing old gracefully isn't good enough for serious calorie restrictors, the CRS is initiating a research study to determine whether humans respond to CR the same way that mice and monkeys do. We welcome you to check out the details:

Take a step toward controlling aging and finding out more!

Welcome to the Calorie Restriction (CR) Society

Our goal is to help people of *all* ages live longer *and* healthier lives simply by:

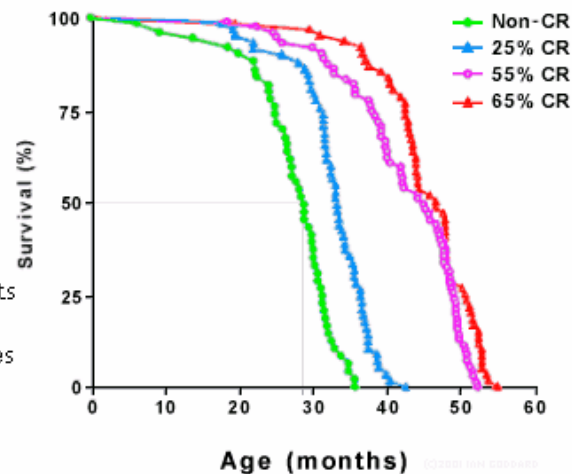
- eating fewer calories
- maintaining adequate nutrition

Since the 1930's extensive scientific research has shown that calorie restricted (CR) diets improve health and **extend lifespans** of nearly every species tested, including worms, spiders, rodents, dogs, cows and monkeys. We believe it is likely that people who **carefully adopt a CR diet** will see similar results.

The CR Society supports the efforts of people who practice CR for future longevity, current health, or other benefits; those curious about or interested in understanding the effects of the diet; and those interested in the development of related, science-based life-extension and health-enhancing technologies. Our **mailing lists** provide a rich forum for such topical discussions.

Calorie Restriction...the only *proven* life-extension method known to modern science.

LIFESPAN OF CR MICE VS NON-CR MICE



Latest Society News

Help support a scientific study on human CR!

New book on CR. Written by CR Society President Brian M. Delaney and Lisa Walford, daughter of the eminent research gerontologist Dr. Roy Walford. Available now!

Recent Media Stories

Live, fast, die old. - East Bay Express newspaper. Interviews with CR Society members, including Warren Taylor, the society secretary. 1/18/2006

Restriction of calorie intake slows down heart aging. Research done by Luigi Fontana. 1/17/2006

CBS Evening News. Interview with Joseph Cordell, CR Practitioner, text & video. 12/30/2005

Restricting diet my reverse early-stage Parkinson's. Research at Oregon Health & Science University. 11/14/2005

PBS NewsHour - Science of Aging. Includes video with



[Home](#)

[About the CR Society](#)

[CR Guide](#)

[Society Services](#)

[Membership](#)

[Video Clips](#)

[Frequently Asked Questions](#)

[Contact Us](#)

Research that will change your life!

The evidence that caloric restriction extends life in animal species inspires thousands to adopt calorie restriction as a way of life. But unlike animals in research studies that have clearly defined diet parameters and protocols, humans have many ideas about how caloric restriction should be practiced.

If you are reading this, most likely you are very serious about functioning at your peak. Nothing is more precious.

But how can you know whether your limited-calorie lifestyle really slows aging? And whether are not you are practicing calorie restriction, how will you know if supplements, exercise, amount of protein intake, hormone replacements or any other of the myriad choices that are persuasively argued for - actually accelerates aging or increases risk of cancer or other serious disease? An objective method of evaluation is vital to avoid life-shortening mistakes.

That's why the **Calorie Restriction Society** has initiated a milestone study that will correlate human calorie restrictors' genetic expression and cell signaling indicators to clinical markers. Once these correlations are established, serious longevists will be equipped with easy-to-run clinical tests that indicate how well their regimens are working.

We've asked several renowned researchers to handle aspects of the project: **Luigi Fontana**, M.D., Ph.D., **Stephen Spindler**, Ph.D., and **Shin-ichiro Imai**, M.D., Ph.D.

But we can't do it without your help.

We need to raise \$230,000 to make this project happen. (**check status**) This means we need contributions large and small. Make an investment that is priceless and learn more about slowing aging - what works and what doesn't. Please send your donation directly to the Society Treasurer,

David R. Stern
7223 S Rt 83 #142
Willowbrook, IL 60527

Or to

Bob Cavanaugh at the Calorie Restriction Society
187 Ocean Drive
Newport, NC 28570

or donate online using a **Credit Card** .

For other payment options, please contact The Calorie Restriction Society by phone at:

914-923-1605

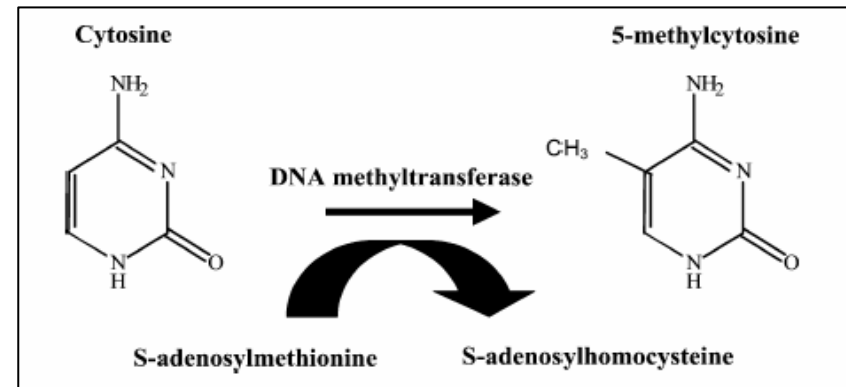
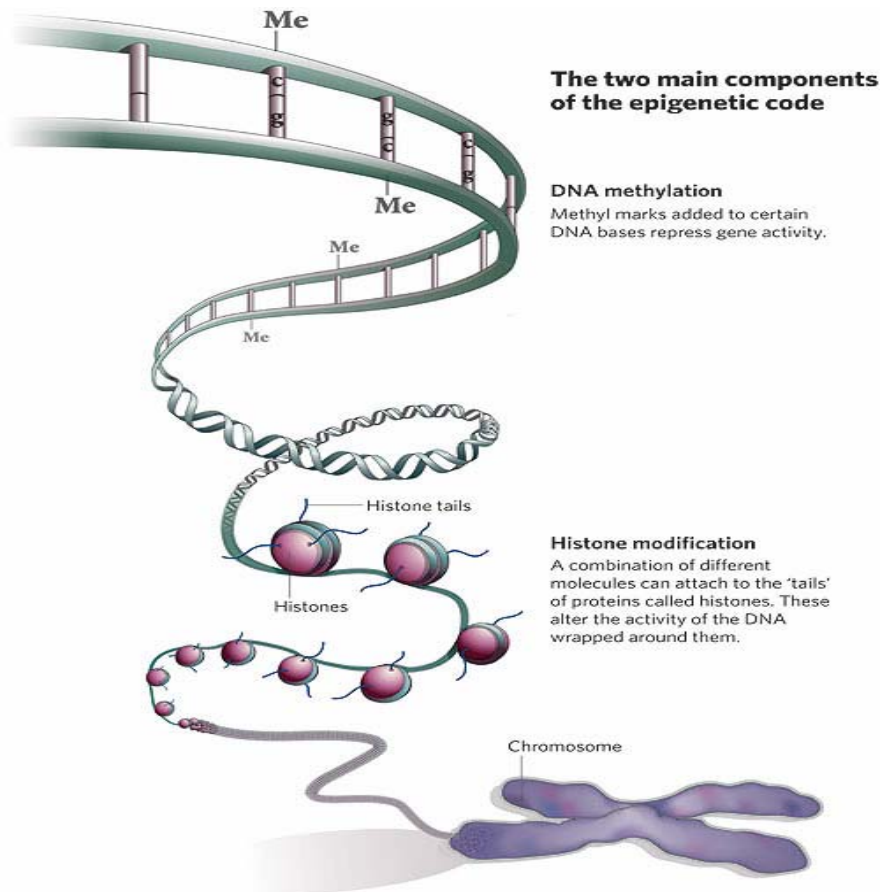
or toll-free at **866-894-1812**

Questions? Please be in touch with **Paul McGlothlin**. Want to know more about the Calorie Restriction Society? Ask me or

Background to project - calorie restriction and ageing

- Well established (based on research going back several decades) that calorie restriction (approximately 60% normal intake in rodents) increases lifespan and/or reduces incidence of ageing-related disease in model organisms including yeast, *C. elegans*, *Drosophila*, mice.
- Applies in humans?
- Molecular basis unknown
 - Reduced metabolic rate leads to generation of fewer, DNA-damaging free radicals?
- Increased expression of the protein Sirt1 appears to be involved
 - Effects on insulin/IGF1 signalling pathway?
 - Increased stress-resistance?
 - Higher threshold for apoptosis?
 - Novel idea - effects of Sirt1 activity on epigenetic modification of DNA??

Background to project - epigenetic modification of DNA



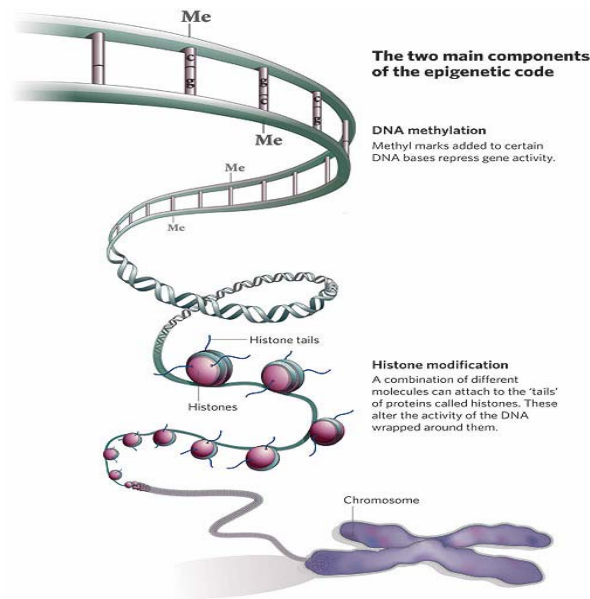
Methylated DNA is associated with deacetylated histones

Background to project - 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.

Background to project - Sirt1 activity relevant to DNA methylation

- Recap - calorie restriction increases expression of Sirt1
- Sirt1 has histone deacetylase activity

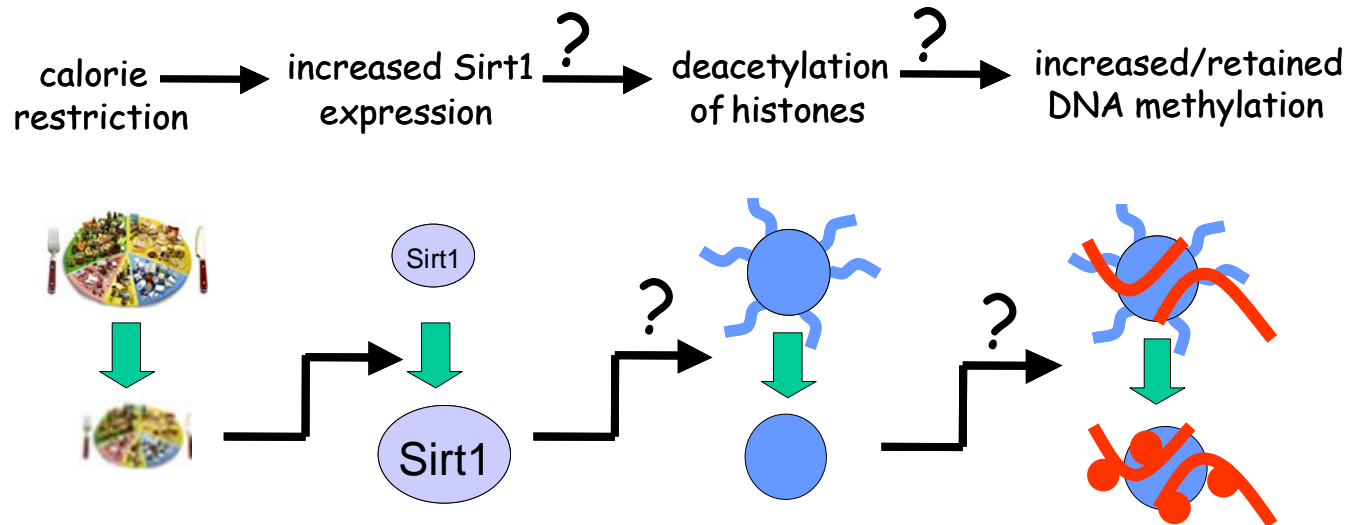


Methylated DNA is associated with deacetylated histones

- So increased Sirt1 expression may affect histone acetylation and hence DNA methylation

Hypothesis

- The beneficial effects of calorie restriction on ageing include maintenance of DNA methylation patterns mediated through increased histone deacetylation by increased activity of the Sirt1 protein.



Aim

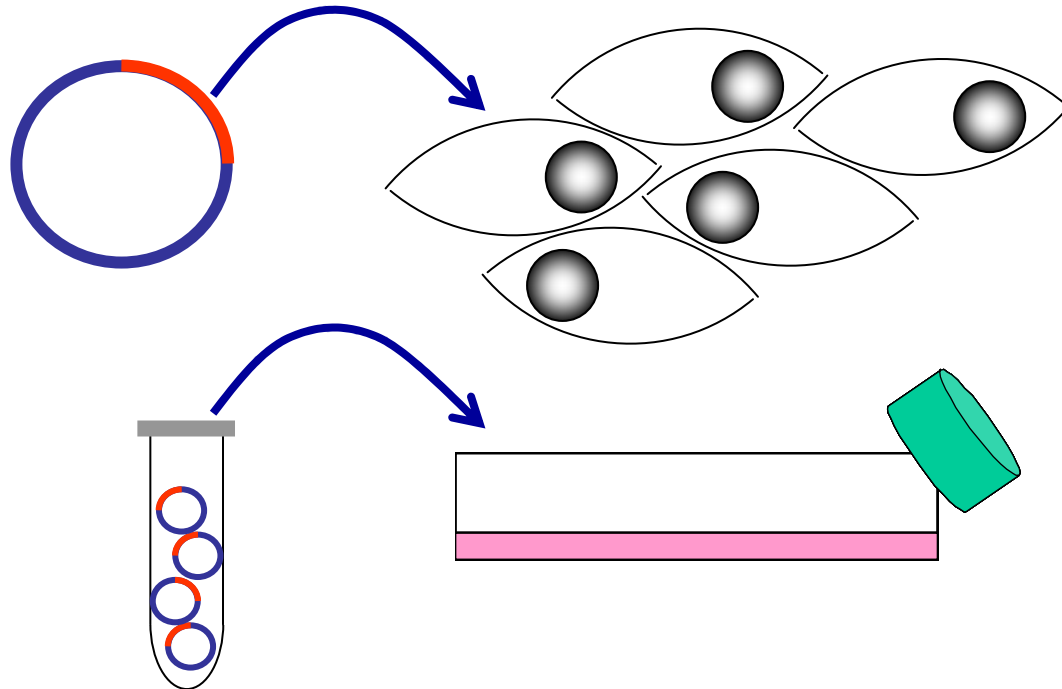
- Establish proof of principle that increased Sirt1 expression can affect DNA methylation.

Approach

- Researcher - Luisa Wakeling
- Express high levels of human Sirt1 in cultured human cells (SW480) from a "transgene" (coding sequence of the human Sirt1 gene introduced into the cells) and measure DNA methylation compared with control cells.

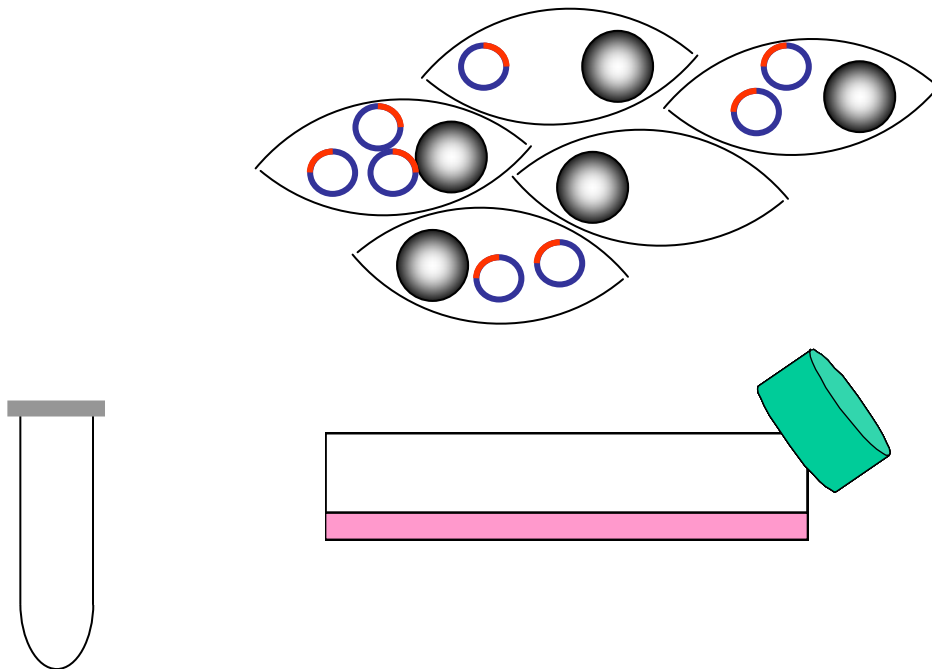
Approach

- Researcher - Luisa Wakeling
- Express high levels of human Sirt1 in cultured human cells (SW480) from a "transgene" (coding sequence of the human Sirt1 gene introduced into the cells) and measure DNA methylation compared with control cells.



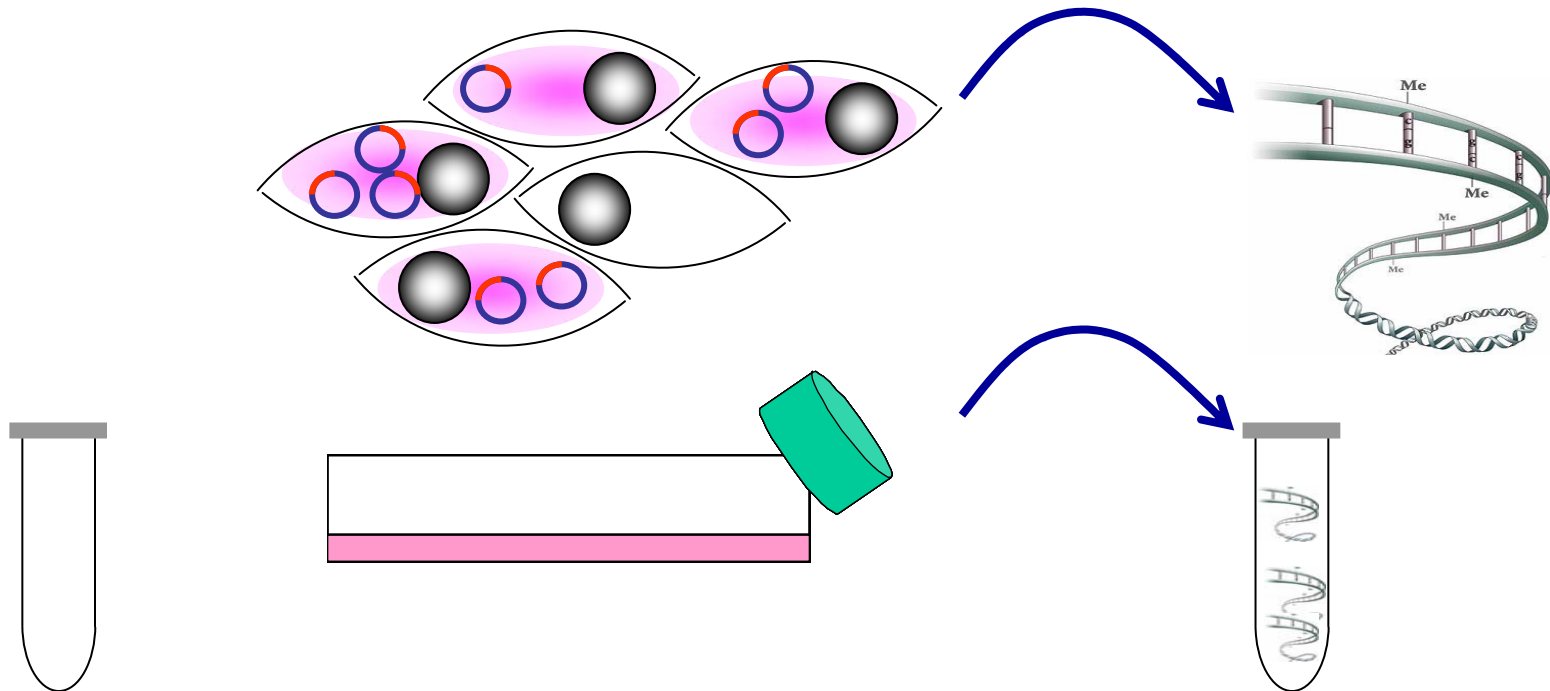
Approach

- Researcher - Luisa Wakeling
- Express high levels of human Sirt1 in cultured human cells (SW480) from a "transgene" (coding sequence of the human Sirt1 gene introduced into the cells) and measure DNA methylation compared with control cells.

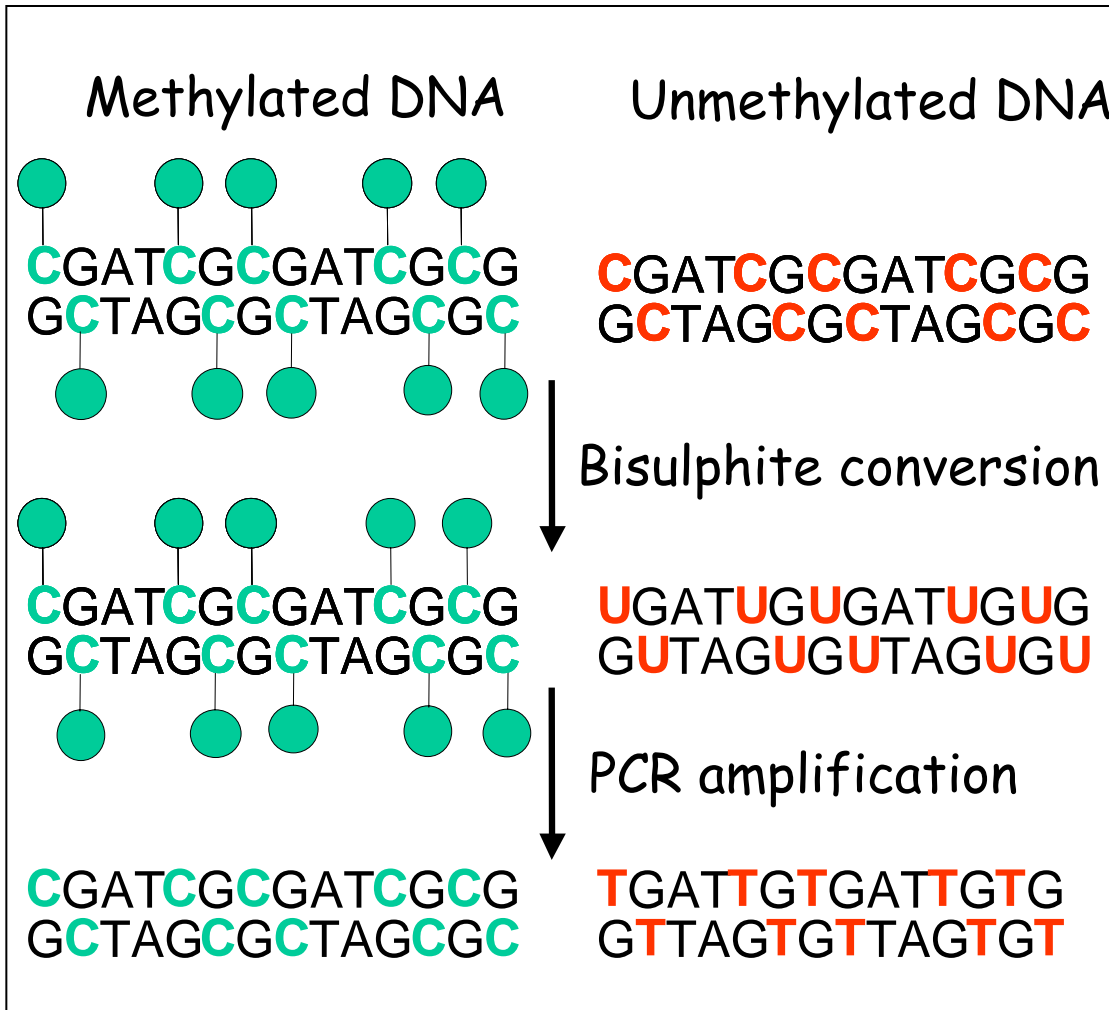


Approach

- Researcher - Luisa Wakeling
- Express high levels of human Sirt1 in cultured human cells (SW480) from a "transgene" (coding sequence of the human Sirt1 gene introduced into the cells) and measure DNA methylation compared with control cells.



Measurement of DNA methylation



Target which sequences?

LINE1 elements

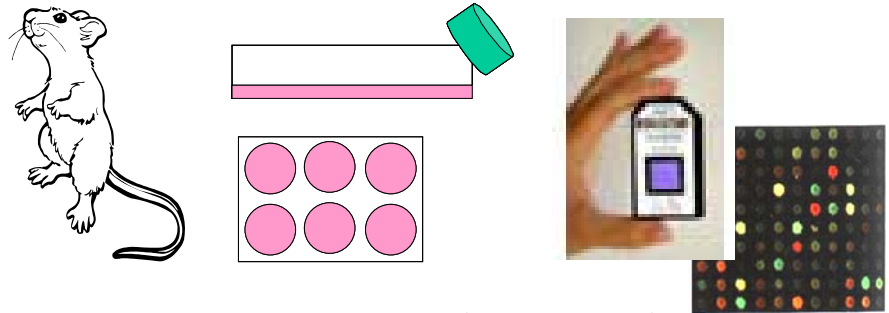
C-MYC

ER

IGF2

GFP reporter gene

Future directions



- Use genome-scanning techniques (e.g. differential methylation hybridisation; ChIP on chip) to identify specific sites in the genome showing altered patterns of DNA methylation and/or altered patterns of histone acetylation in response to increased Sirt1 expression (in cells; BBSRC DTG Studentship from ICaMB) or calorie restriction (in ageing mice).
- Determine effects of such methylation patterns on target gene expression.
- Increase/knockdown expression of target genes in model organisms (*C. elegans*, mice?) whose expression is modulated by methylation and measure effects on ageing to identify gene targets of calorie restriction that can affect the ageing process.

Acknowledgements

- Professor John Mathers
Newcastle University



- Luisa Wakeling
Newcastle University

