Analyzing the Relationship Between Oxidative Stress and Aging in Drosophila

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Glutathione-dependent defence against ROS

External factors lead to the production of reactive oxygen species (ROS) which can cause damage to macromolecules. The release of reactive molecules results in detoxification, protecting cells from oxidative stress. Mitochondria play a crucial role in this process.
• GCL catalyses the rate-limiting step in glutathione biosynthesis
• Activity is inhibited by glutathione at physiological concentrations
Why is glutamate cysteine ligase (GCL) potentially important with regard to ageing?

• It is a critical regulator of Glutathione, the ‘master antioxidant’.
• Glutathione is an endogenously synthesised antioxidant which is of central importance in regulating levels of reactive oxygen species and environmental toxins in cells.
GCL in mammals

- Holoenzyme comprises catalytic (GCLC) and modifier (GCLM) subunits
- GCLC has catalytic activity but the holoenzyme is:
  - catalytically more efficient
  - less susceptible to inhibition from GSH
- Non-mammalian GCLM homologues not identified until recently
- Kinetics suggest it may not be required for efficient catalysis in some species
Do levels of glutathione influence life span?

- We wished to test the hypothesis that an increased capacity to synthesise glutathione would extend life span, using *Drosophila* as a model system.
- Alteration of glutathione levels would be achieved by modulating GCL activity.
- This first required the molecular characterisation of GCL in *Drosophila*…….
Molecular characterisation of *Drosophila* GCL

- DmGCL is a heterodimer comprising a catalytic (DmGCLC) and regulatory (DmGCLM) subunit
- Transcription of DmGCLC is induced by oxidative stress
- DmGCLM modifies activity of DmGCLC in a similar way to the mammalian form
- Disulphide bridges form between DmGCLC and DmGCLM
- The cysteine residues on DmGCLM that could interact with DmGCLC were identified using iodoacetamide modification
- DmGCLM lacking the critical cysteine residues does not form covalent linkages with DmGCLC under non-reducing conditions.
The cysteine residues in DmGCLM that could interact with DmGCLC identified by iodoacetamide modification

DmGCLM lacking the critical cysteine residues A B and D fails to establish covalent linkage with DmGCLC
Elevating GSH titre by transgenic overexpression of DmGCL

- Technology for transgenic expression well established in *Drosophila*
- We have used the Gal4-UAS system
  - Wide selection of P elements expressing the transcription factor Gal4 in specific spatiotemporal patterns
  - Gal4 drives expression of transgene via the UAS
- Can mix and match Gal4 drivers with UAS responders
- **Cautionary notes:** the expression patterns of drivers may be unexpected; effects on complex traits may be masked by genetic background
**tubGal4 drives overexpression of pUASTGclc**

**Diagram:**
- **tub-Gal4** drives overexpression of **pUASTGclc**.
- **DmGCLC** and **DmGCLM** proteins are shown in the image.

**Graph:**
- pmol GSH per fly, 95% CI
Ubiquitous overexpression of GCL does not extend lifespan

% survival

days

UAS-GCLM, n=121
TubGal4>UAS-GCLM, n=178
TubGal4, n=150
UAS-GCLC, n=186
TubGal4>UAS-GCLC, n=199
Ubiquitous overexpression of both subunits of GCL is lethal

TubGal4>UAS-GCLM

%Survival relative to 3rd instar

3rd Instar Pupae Adults

TubGal4>UAS-GCLC

3rd Instar Pupae Adults

TubGal4>UAS-GCLC, UASGCLM

3rd Instar Pupae Adults

TubGal4>UAS-GCLC, UASGCLM-ABD

3rd Instar Pupae Adults
Gclm<sup>L0580</sup> is a strongly hypomorphic allele

- DmGclmL0580 is an insertional allele, and is strongly hypomorphic.
- Loss of DmGCLM did not substantially increase sensitivity to oxidative stress caused by diethylmaleate, nor did it appear to significantly shorten lifespan under the conditions used.
The GeneSwitch-UAS system

responder

UAS ORF stop marker

gene product

RU486

GeneSwitch stop marker

driver
Overexpression of GCL in a pan-neural pattern can extend lifespan

Statistically significant extension of lifespan in males, but not in females
Summary

- DmGCL appears to be regulated in a similar manner to mammalian GCL
- Identified cysteine residues as candidates for interaction with DmGCLC
- Overexpression of DmGCL leads to elevation of GSH titres
- Overexpression of DmGCLC reduces viability
- Overexpression of DmGCL in neural tissues extends lifespan in males
- Loss of DmGCLM reduces GSH titre but does not affect OS resistance or lifespan
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