Radiation Effects on Metabolic Gene Expression

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About Calvin

- From Eden Prairie MN
- Bethel University, St. Paul MN
  - Chemistry and Biochemistry
- Enjoy the outdoors
  - Camping
  - Skiing
- Electronics!

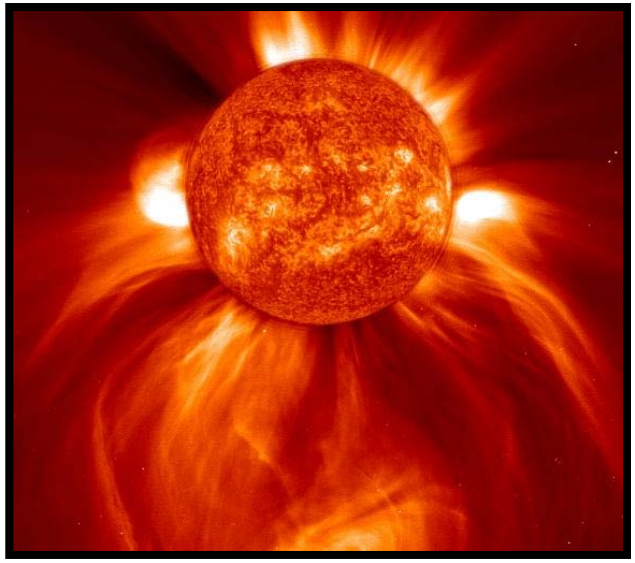
Space Life Sciences Directorate

- Mission: *"To optimize human health and productivity for space exploration"*

- Human Adaptation and Countermeasures Division
  - Understand the normal human response to space flight
  - Develop countermeasures to protect crew health

Radiation

- Sources:
  - Galactic cosmic rays
  - Solar particle events
- Concerns:
  - DNA damage
  - Increase in oxidative products
- Health outcomes:
  - Cataracts
  - Cancer
  - Central nervous system damage
  - Radiation sickness
  - Unknowns



Pharmacology

- Medications are vital to human spaceflight
- Spaceflight induces changes in human physiology
- Yet drug metabolism is assumed to be the same as on earth
  - Is this assumption valid?

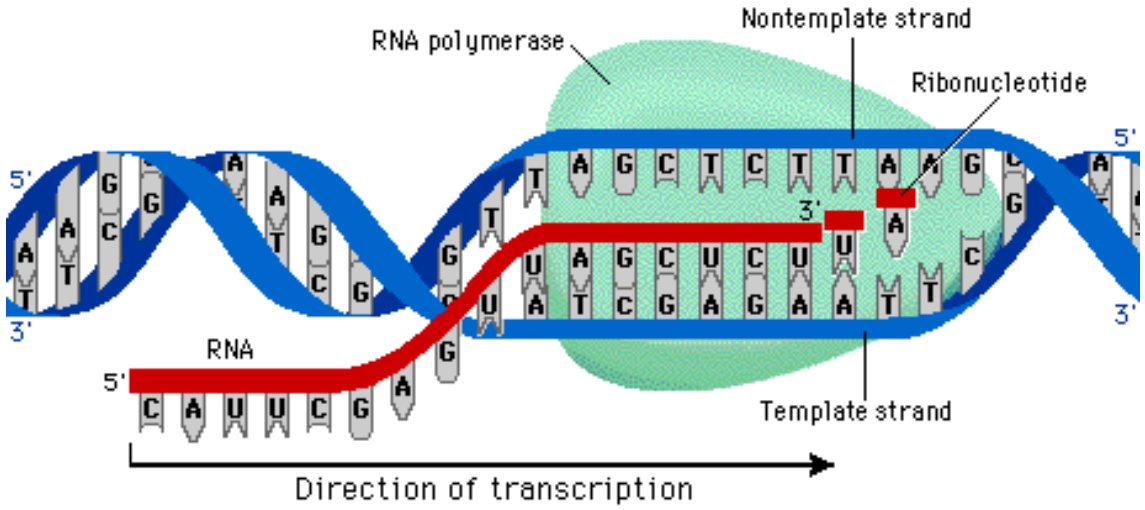
Research

- Challenge of spaceflight: **Radiation**
- Application: **Drug Metabolism** in the liver

- In General, activity of liver metabolic enzymes determines the concentration of circulating drugs
  - Decreased liver function = drug overdose
  - Elevated liver function = ineffective treatment

Goal

- Identify how radiation exposure affects transcriptional gene expression in the liver
  - Focus on genes associated with:
    - Drug Metabolism
    - DNA Repair
- Foundation for implementing countermeasures



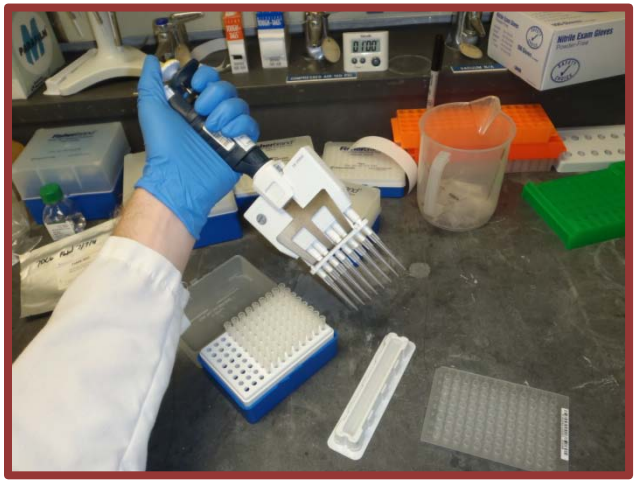
Method Overview

RNA Extraction & Purification

Liver Tissue → **RNA**

Reverse Transcription

→ **cDNA**



RT-qPCR

→ **Gene Expression**

Methods: Ground Model

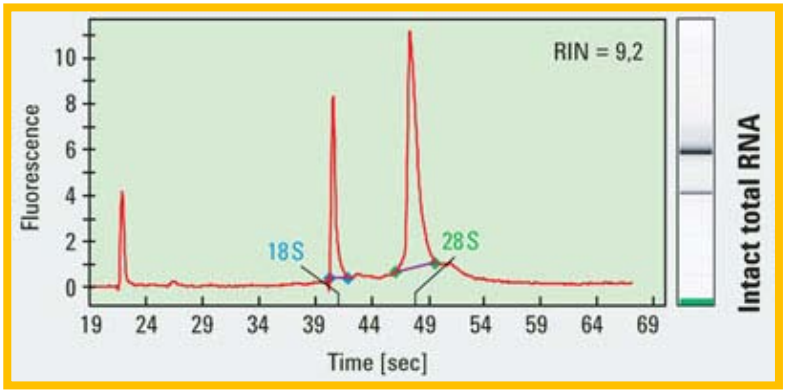
- Male C57 mice exposed to ^{137}Cs in 4 Groups :
  - Control
  - Low dose (50 mGy)
  - High dose (6 Gy)
  - Both radiation doses (low then high) separated by 4 hours
- Each group contained 6 mice
- 4 sets sacrificed at 4 hours, 24 hours, 7 days and 13 days after their last radiation exposure
  - I worked with the 7 day set
- Livers flash frozen in liquid nitrogen

Methods: RNA Extraction

- Agilent Absolutely RNA Miniprep Kit
  - Tissue homogenized in a lysis buffer
  - Prefiltered sample in a spin cup
  - RNA-binding spin cup
    - Series of washes remove DNA and proteins
  - Highly pure RNA is eluted into a microcentrifuge tube using an elution buffer

Methods: RNA Quality Testing

- Agilent 2100 Bioanalyzer with Agilent RNA 6000 Nano kit
  - Microfluidic chip
- Check total RNA integrity and concentration
- RNA integrity number (RIN)
  - Samples must have RIN > 8

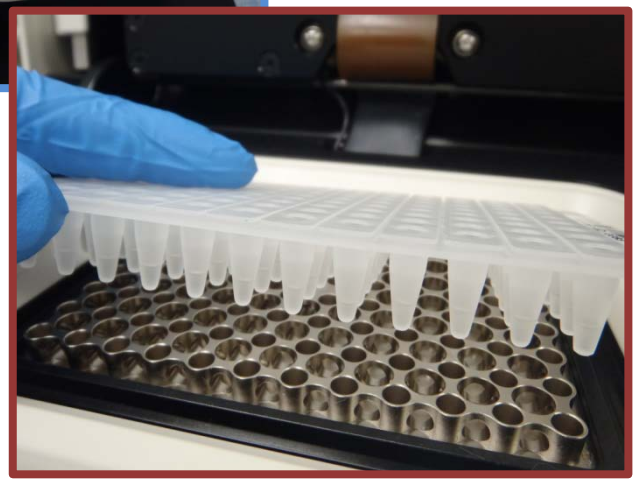


Methods: Reverse Transcription

- SABiosciences RT2 First Strand Kit
  - cDNA prepared from RIN > 8 RNA samples
- PCR requires DNA
  - In vitro transcription by mRNA and enzymes:
    - Reverse transcriptase and DNA polymerase

Methods: RT-qPCR

- Real-time polymerase chain reaction
- Measure gene expression
- SABiosciences RT2 Profiler Arrays
  - DNA Repair and Drug Metabolism
  - Test many genes simultaneously
  - 96-well plates
    - 84 pathway focused genes
    - Controls for testing inter-well and intra-plate consistency
  - SYBR Green Detection

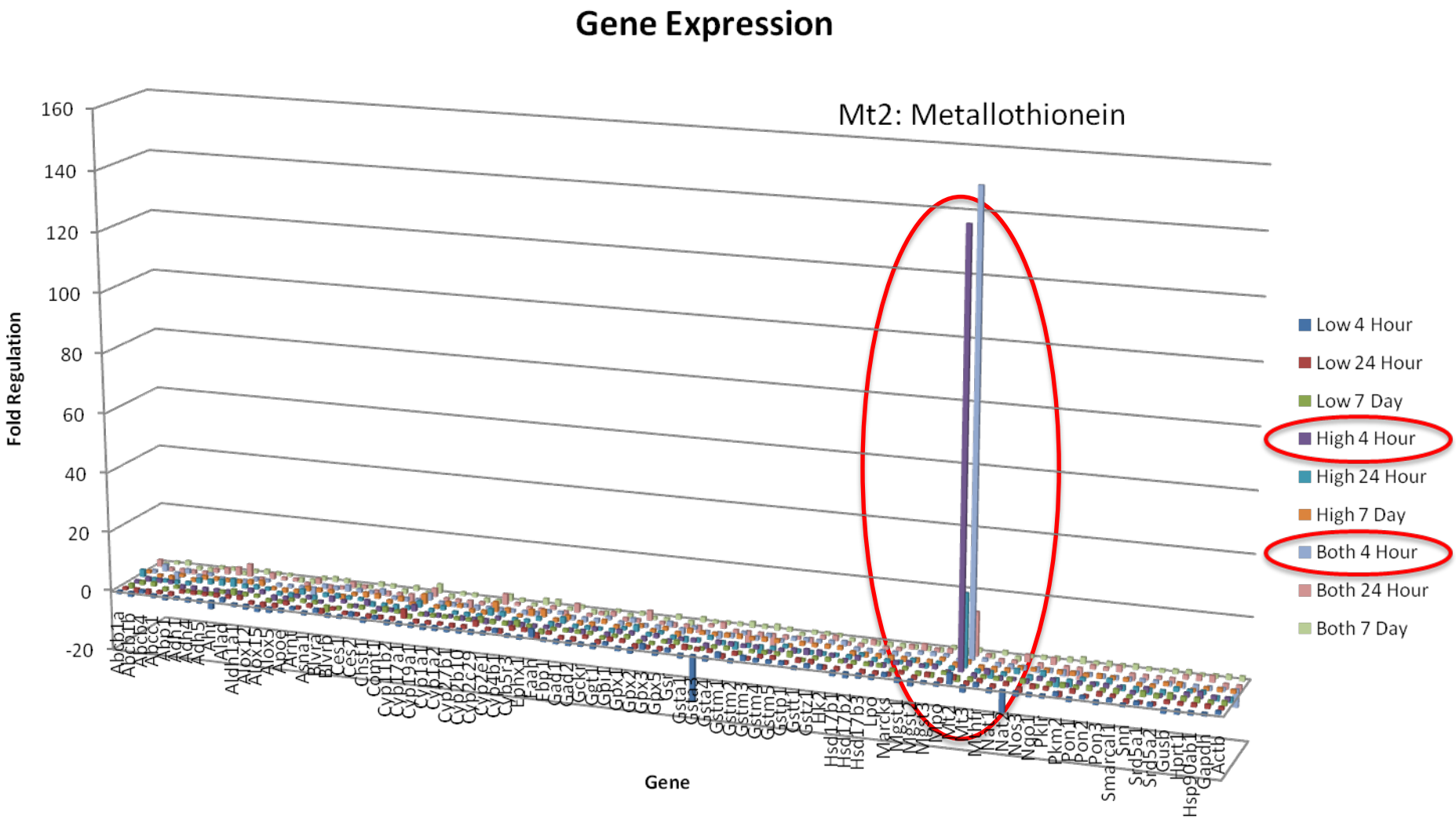


Calculations

- Analyzed C_t data from all data sets
  - 72 mice × 89 genes per plate × 2 plates
    = 12,816 genes total
- Set RT-qPCR baseline at 600
- Determined gene expression by $\Delta\Delta C_T$ method
- Normalized data to housekeeping genes
  - Drug Metabolism: Adh1, Blvrb, Gstm4, Gstm5, Marcks, Snn
  - DNA Repair: Polb, Rad21, Rpa3, Slk, Tdg, Xrcc4

$$\frac{\frac{2^{-\Delta C_t(GOI)}\,\text{expt}}{2^{-\Delta C_t(HKG)}\,\text{expt}}}{\frac{2^{-\Delta C_t(GOI)}\,\text{control}}{2^{-\Delta C_t(HKG)}\,\text{control}}} = \frac{2^{-[C_t(GOI)\,-\,C_t(HKG)]}\,\text{expt}}{2^{-[C_t(GOI)\,-\,C_t(HKG)]}\,\text{control}} = \frac{2^{-\Delta C_t}\,\text{expt}}{2^{-\Delta C_t}\,\text{control}} = 2^{-\Delta\Delta C_t}$$

Drug Metabolism Results



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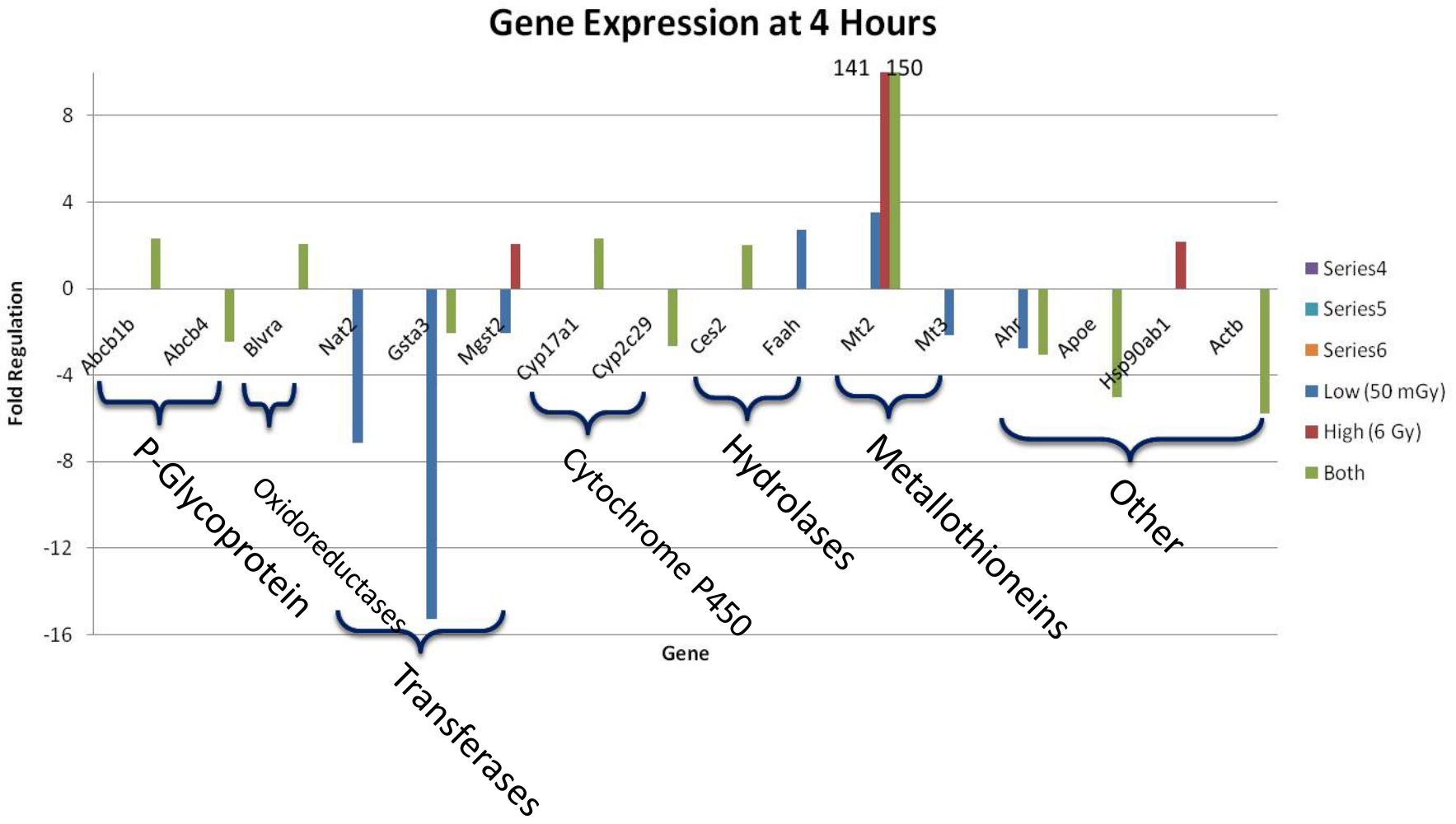

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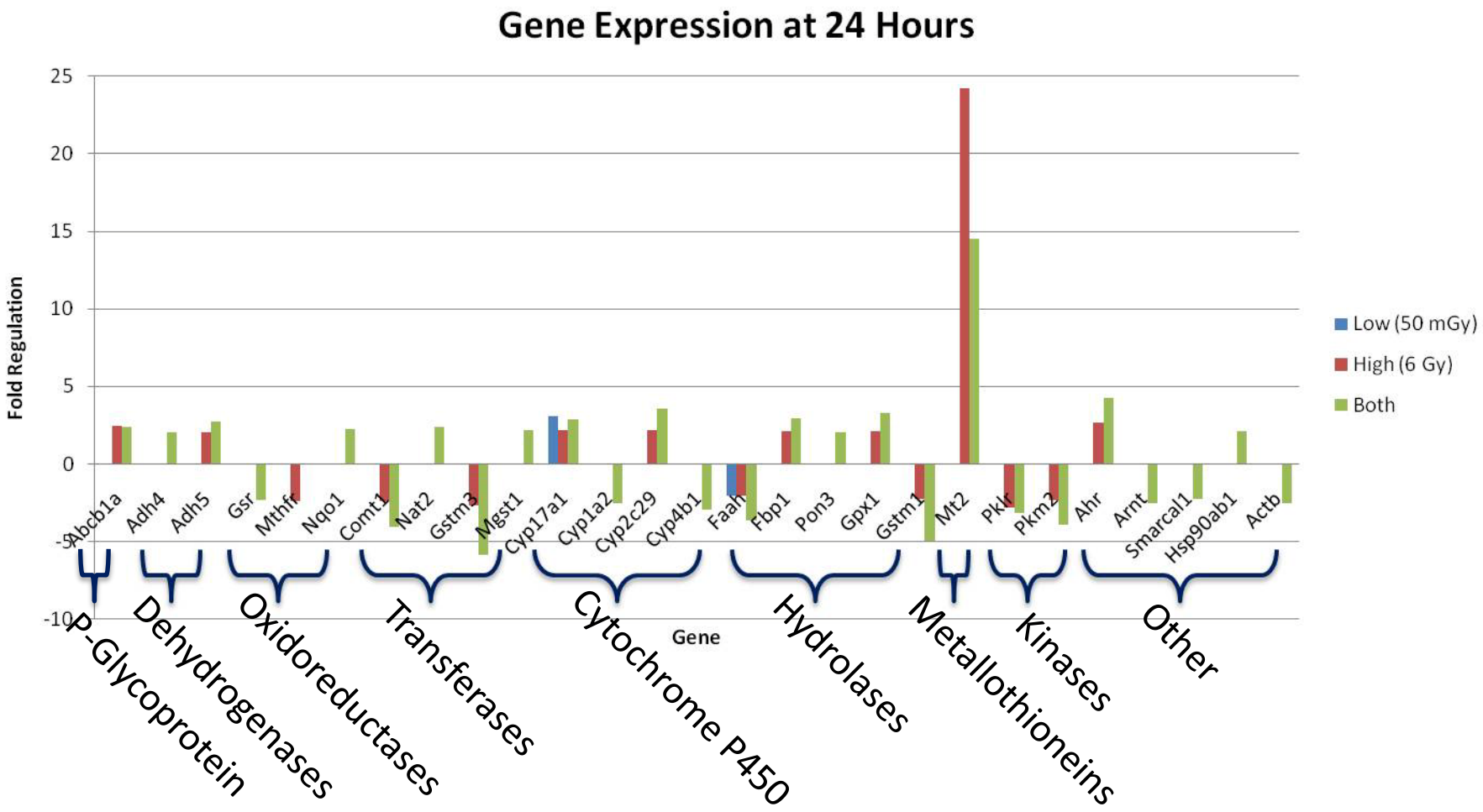

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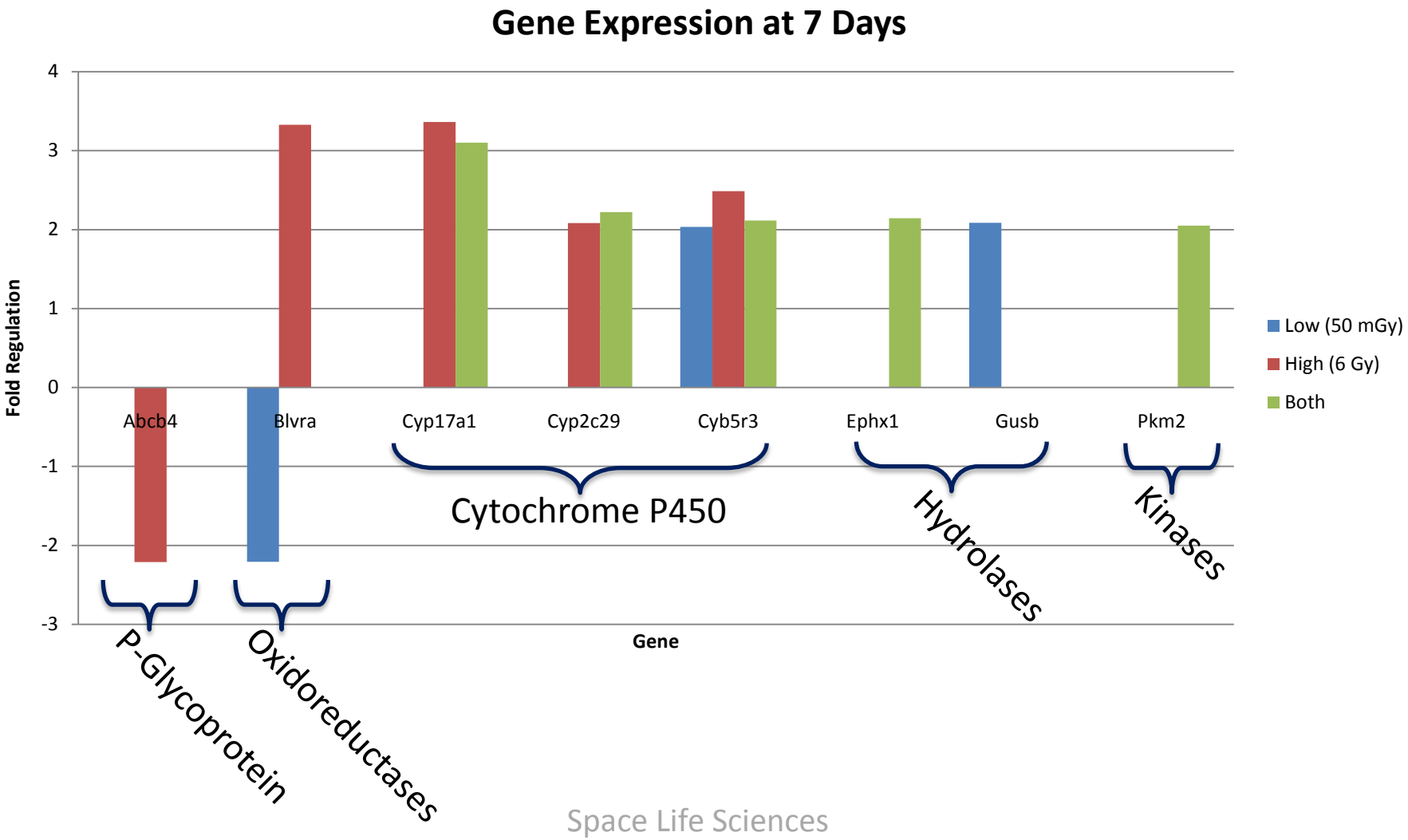
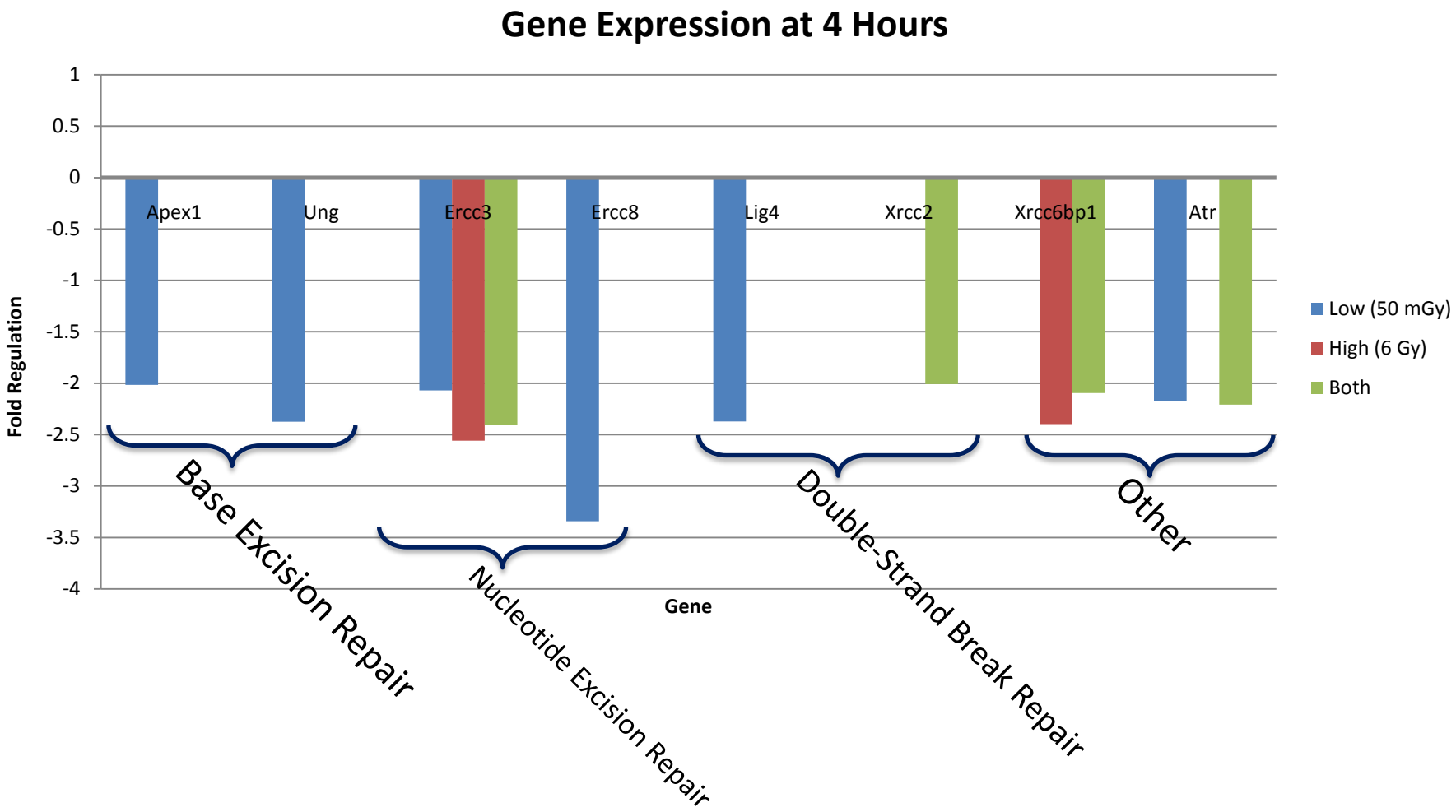


DNA Repair Results



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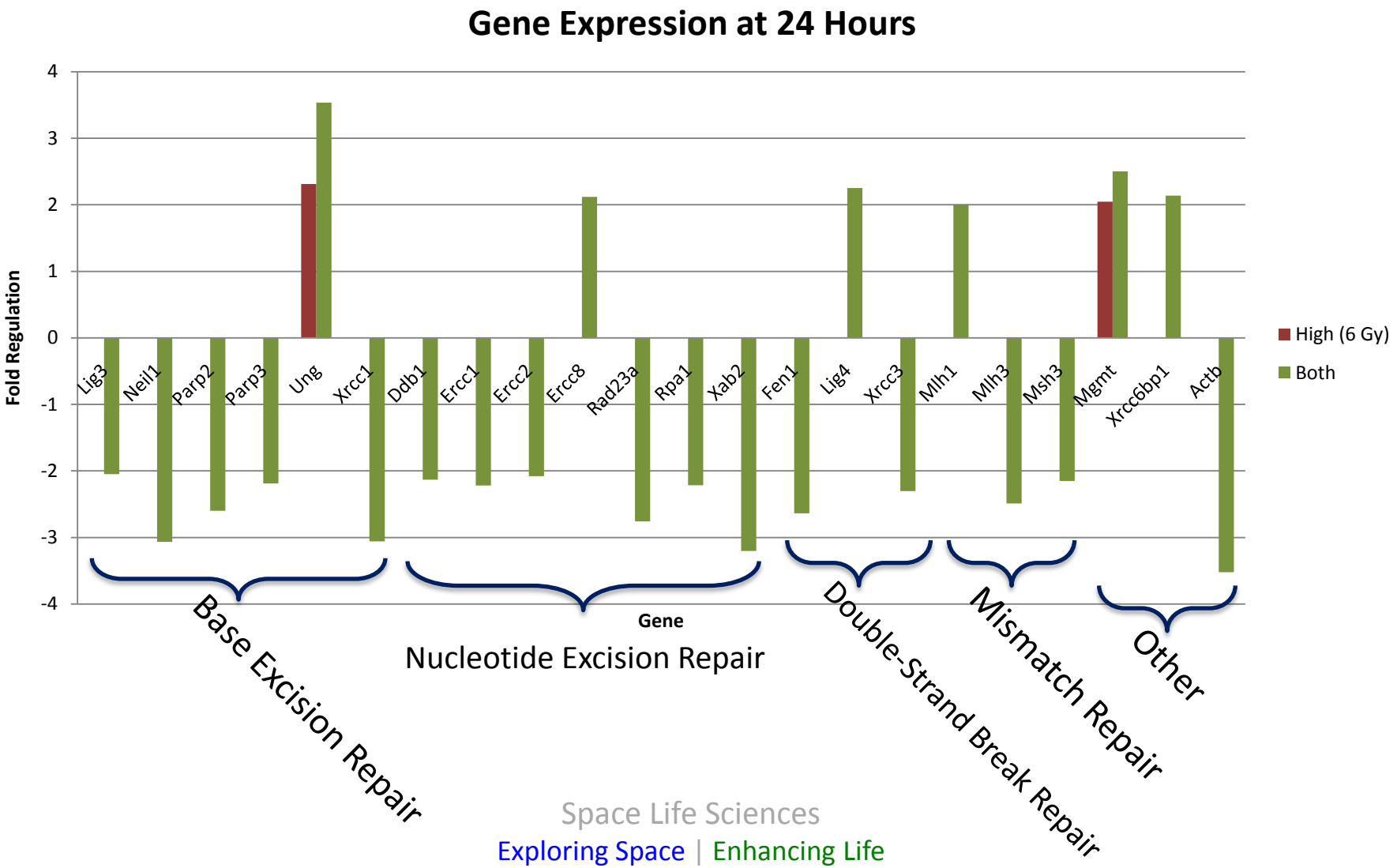

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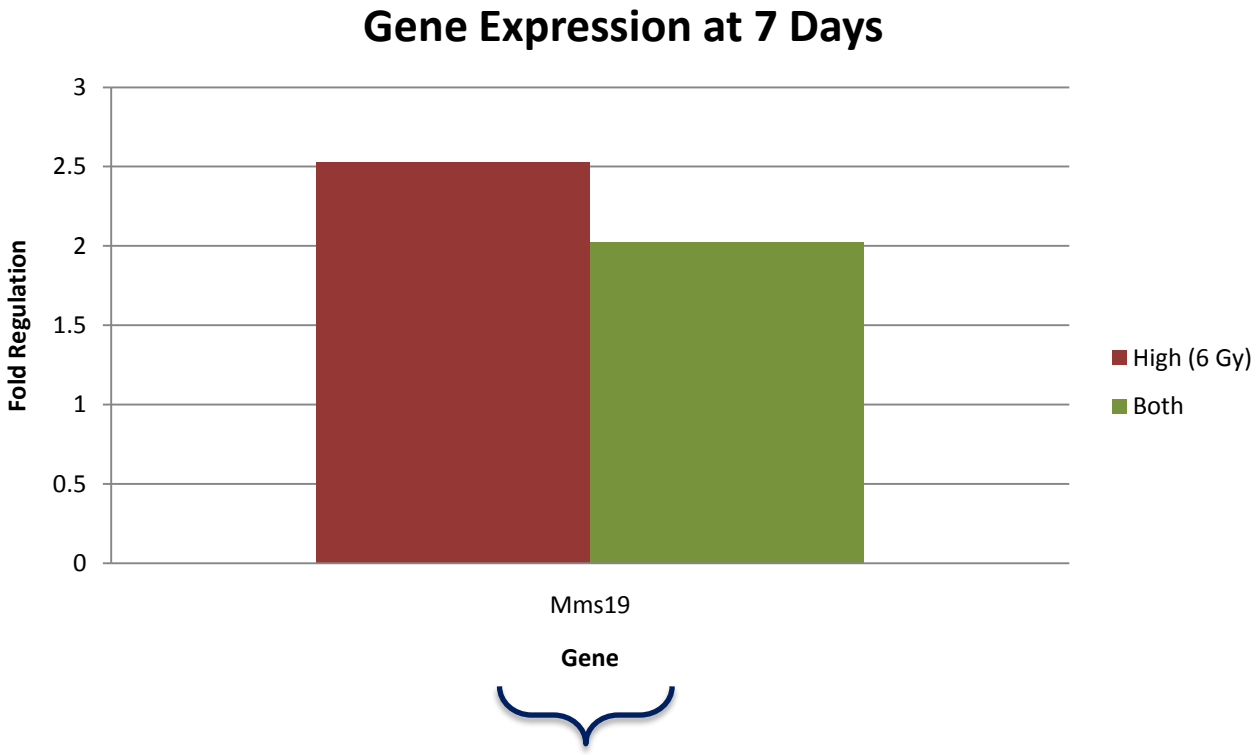
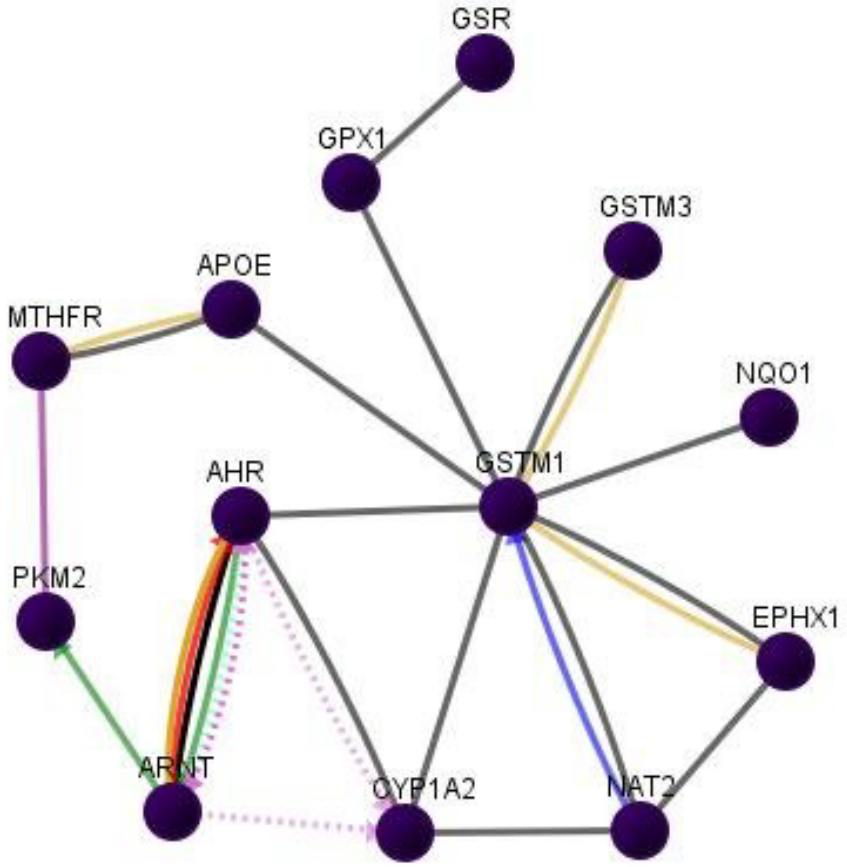


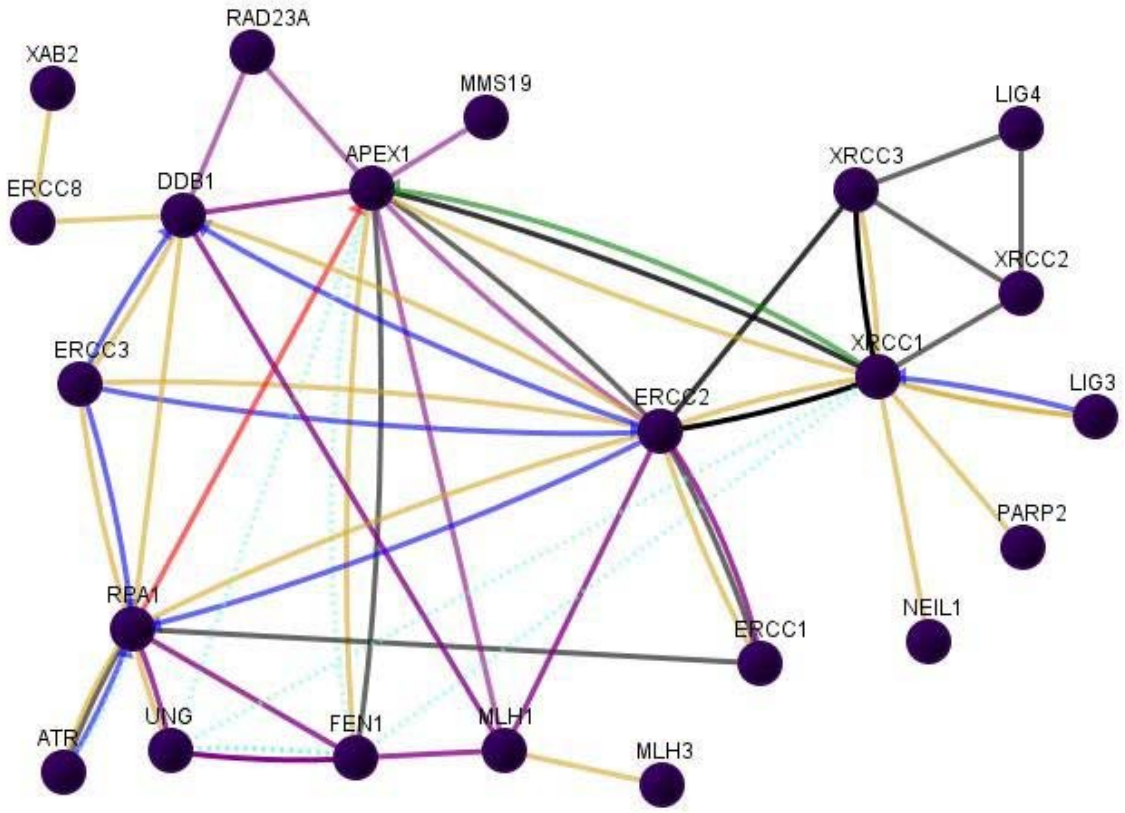
Nucleotide Excision Repair

Drug Metabolism Gene Relationships



- Down-regulation
- Up-regulation
- Regulation Direction Unknown
- Coexpression
- Chemical Modification
- Physical Interaction
- Predicted Protein Interaction
- Predicted TFactor Regulation
- Other

DNA Repair Gene Relationships



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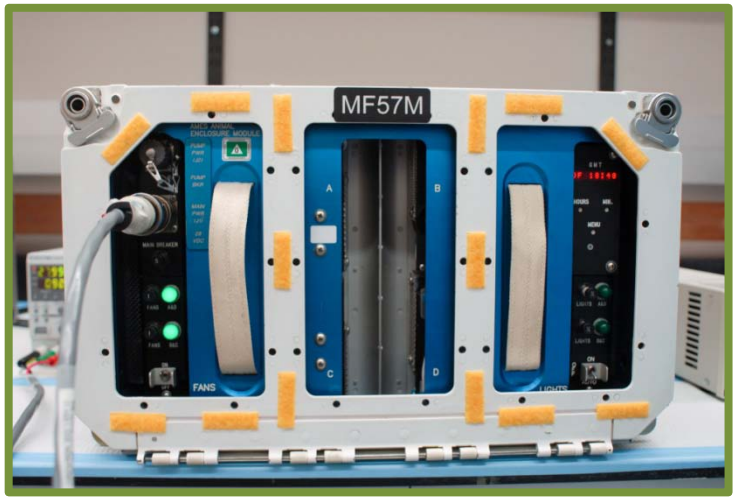
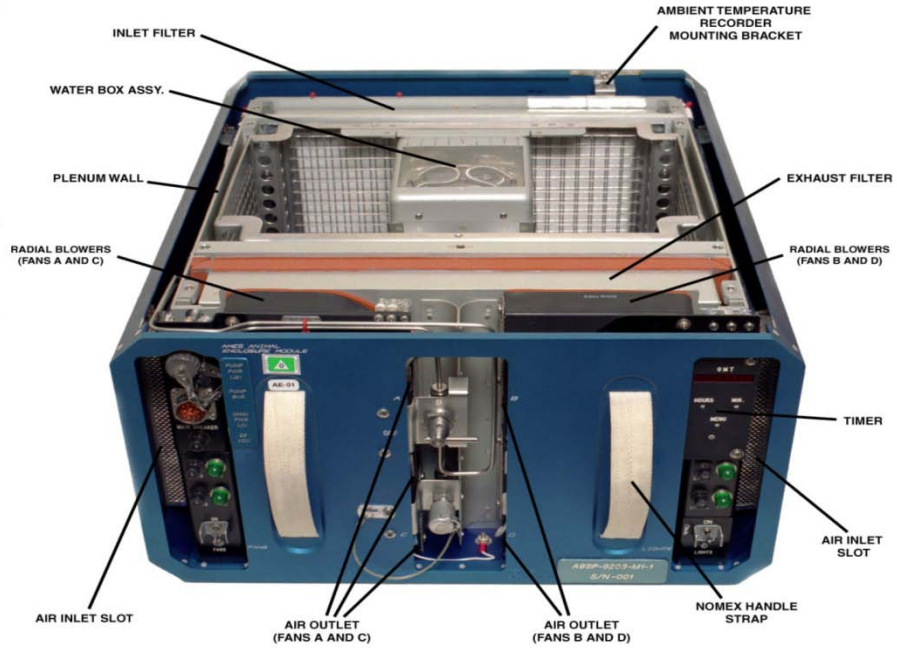
Conclusions

- The expressions of 65 genes have been found to be affected by radiation exposure in mice
  - Effects vary with time and dose
- Radiation exposure effects metabolism of drugs with lipid or steroid hormone-like structures

Methods: Flight Samples

- 15 mice on STS-135!!!
  - 15 controls (Calcein)
  - 15 Baseline (2 weeks younger, no Calcein)
- Processed 6 control and 6 baseline samples
- Legal issues with flight samples….tbd
- Animal Enclosure Module (AEM)





Future Research

- Complete 13 Day and STS-135 data sets
- Evaluate altered genes at protein level
- Correlate findings with drugs used in spaceflight
  - Inform countermeasures

Sources

- V. E. Wotring, L.S. Mangala, Y. Zhang, H. Wu; *Radiation Exposure Alters Expression of Metabolic Enzyme Genes in Mice;* IAA Abstract; 2010
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Thank You!