

Hypergravity Induced Gene Enrichment by Subtractive Hybridization

David Sultemeier

Department of Biology

New Mexico State University

Advisor: Dr. E.E. Serrano



Hypergravity Research

- My lab is interested in how exposure to altered gravity may affect the development of the inner ear and the nervous system
- We are specifically interested in how chronic acceleration will affect the vestibular system during development of the inner ear
- We did a 3 yr study using long arm centrifuges at NASA Ames to provide the hypergravity stimulus
- We are presently analyzing control and hypergravity treated specimens for changes in gross morphology, gene expression and cellular structure


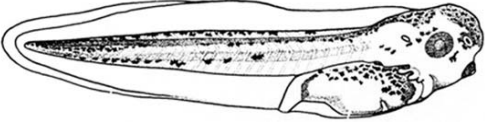
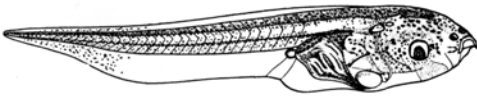
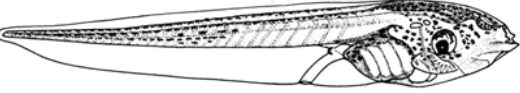
Why *Xenopus*

- Previous studies have demonstrated *Xenopus laevis* adapts well to hypergravity
- Development is well characterized (Kay et. al, 1991)
- *Xenopus* larvae have developed successfully in microgravity (Black et. al, 1996)



Black, S., Larkin, K., Jacquemotte, N., Wassersug, R., Pronych, S., and Souza, K. (1996) Regulative development of *Xenopus laevis* in microgravity. *Adv. Space Res.* 17: 209-217.

Kay, B. and Peng, B., Eds. (1991) *Methods in Cell Biology: Xenopus laevis: Practical Uses in Cell Biology* Academic press Inc. San Diego CA.

	Stage	Length (mm)	Features
	28	3.8-4.0	Length and facial features determine stage > 1 day
	41	6.7 - 7.5	Cement “hang” stage ~3 days
	47	12 - 15	Vestibular system has developed ~5 days
	50	20 – 27	Auditory system begins to develop ~15 days

My research objective

- Isolate genes selectively induced by gravity
 - whole larvae
 - isolated ears (effects on vestibular system)
- Precedents
- Approach my goal by subtractive hybridization

Why am I creating a subtractive cDNA library?

- **Method to enrich differentially expressed genes of two populations in a library**
 - cell type, growth phase, diseased states...
 - AKA “positive selection”
- **What can be done?**
 - screen to find novel or known genes associated with a condition
 - use subtractive library for microarray analysis

NASA AMES 2001 Overview

- **24 ft centrifuge was used to expose stage 28, 41, 46 and 50 *Xenopus laevis* to a gravitational force of 2.0g or 3.0g together with OC and 1.0g controls**
- **Centrifuge was stopped every 2 days for animal care, observation and collection**
 - Animals were staged according to Nieuwkoop and Faber (1966)
 - Animals at important developmental stages were collected for histology, RNA and confocal imaging analysis

NASA AMES 2002 Overview

- **48 ft centrifuge was used to expose stage 28, 41, 45, 47 and 50 *X. laevis* to a gravitational force of 4.2g**
- **Centrifuge was stopped every 2-3 days for a maximum of 90 min for animal care, observation and collection**

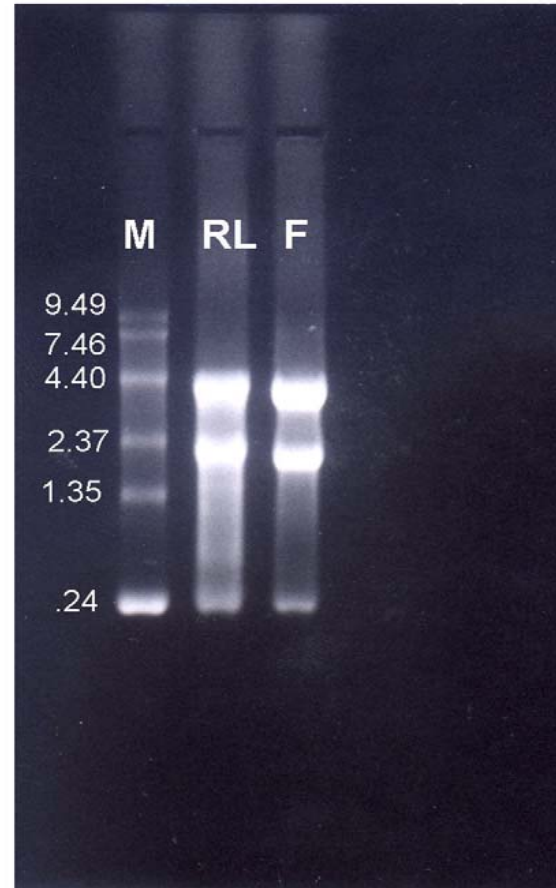
As in year 2, animals were collected for gene expression and morphological analysis

Preliminary Results of NASA AMES

- **Xenopus exposed to gravitational forces of up to 4.2g developed similarly to those of 1.0g**
- **Survival rates of Xenopus at 4.2g, 3.0g, 2.0g, 1.0g and those of the OC were similar**
- **At 4.2G some (10-30%) of the specimens at stages 28 and 50 showed looping and disoriented behavior after 5-10 days of centrifugation**

Approach

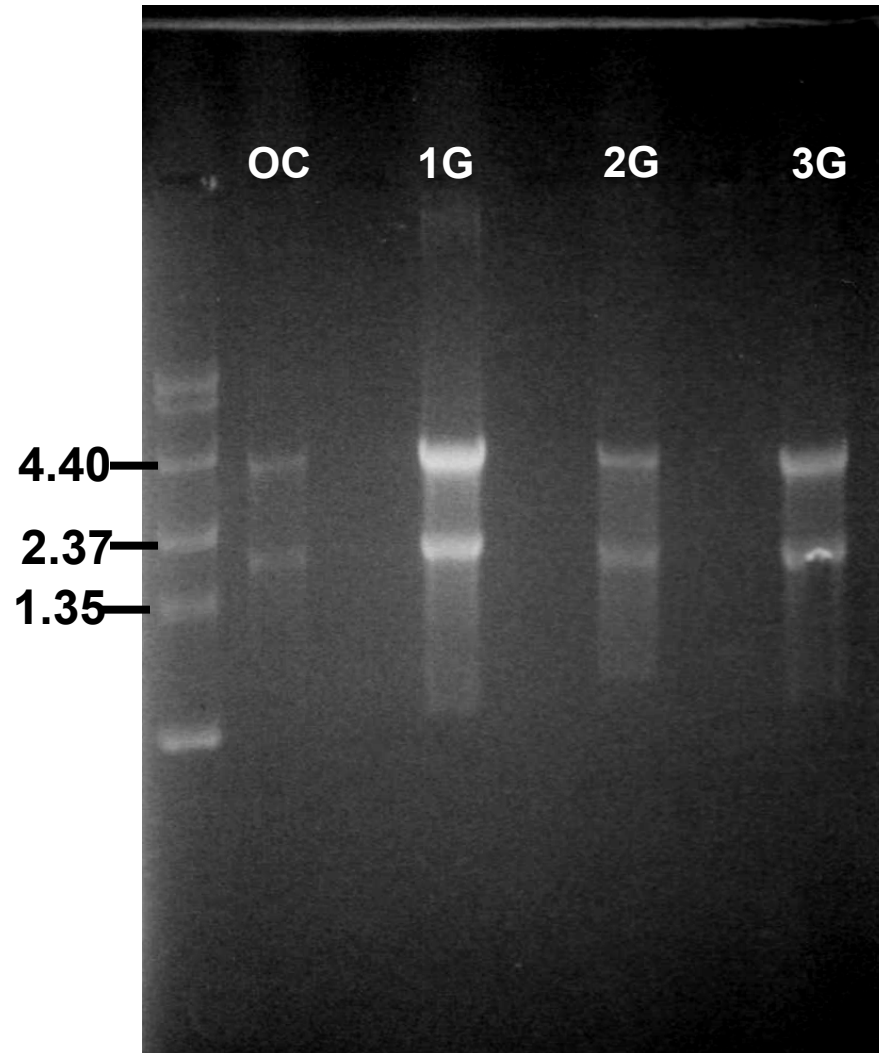
- **Samples have been collected**
- **Practice RNA isolation techniques**
- **cDNA library methods established (Serrano et. al, 2001)**
- **PCR based approaches when starting tissue is small (2 μ g RNA)**
276 ng RNA/ear
- **Prepare RNA and then begin hybridization and cDNA library synthesis**



The gel compares 5 μ g of RNA from NASA samples (RL) with 5 μ g of total RNA isolated from “fresh” larval animals (F) at NMSU. M: Gibco -BRL RNA ladder

Condition	Start (St.)	End (St.)	Days	Samples
1.0G	28	42/43	2	24 Whole
2.0G	28	42	2	25 Whole
3.0G	28	42/43	2	25 Whole
2.0G	28	46/47	8	9 Whole
3.0G	28	47	8	10 Whole
1.0G	28	47/48	11	14 Whole
2.0G	28	47/48	11	10 Whole
OC	28	47/48	11	4 Whole

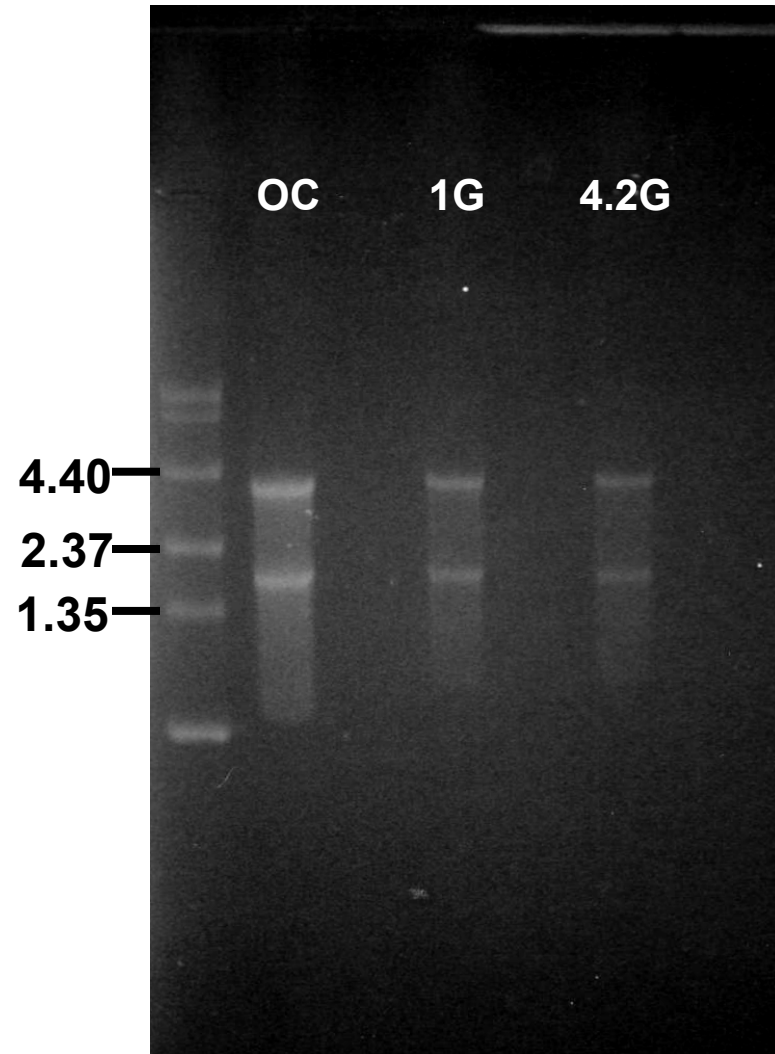
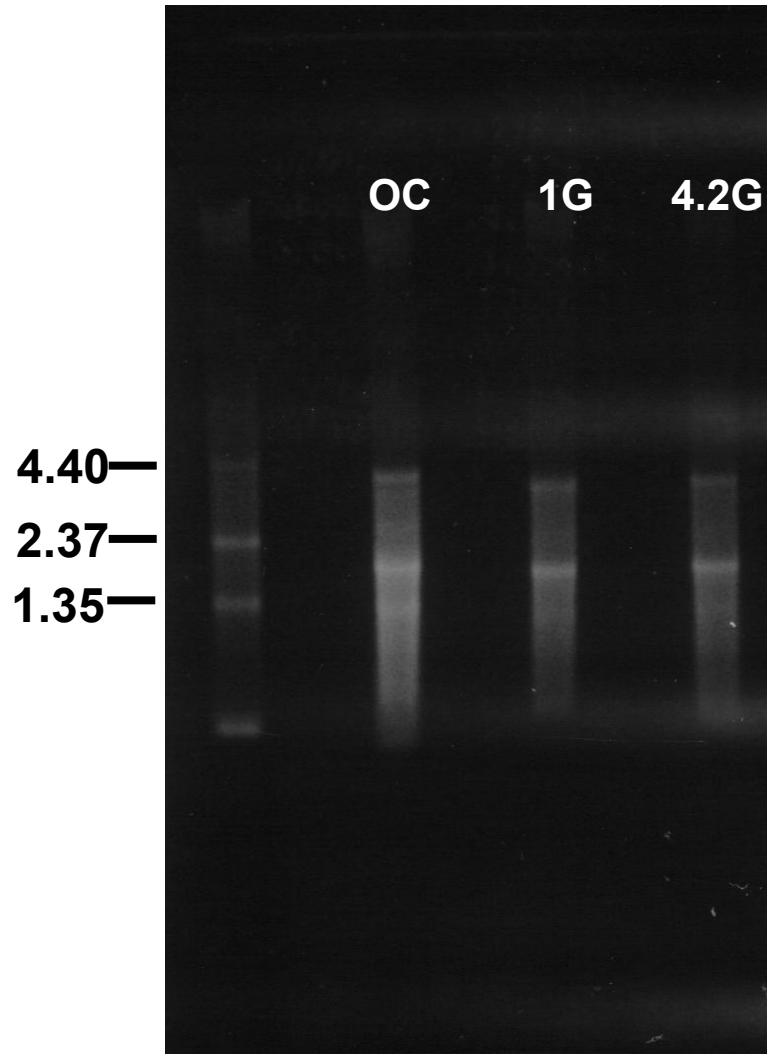
Year 2 Total RNA Has High Integrity



NASA 2001 Total RNA Extraction

Condition	Start (St.)	Start (mm)	End (St.)	End (mm)	Hours	Samples
4.2G	50	22.6	50	24.2	364.3	8 Ears
1.0G	50	22.6	50	25.5	364.3	8 Ears
OC	50	22.6	50	22.6	364.3	8 Ears
4.2G	26	3.3	47	13.0	357	4 Whole
OC	26	3.3	47	12.7	357	11 Whole
4.2G	28	4.2	47	12.3	171.8	6 Whole
1.0G	28	4.2	47	12.9	171.8	4 Whole
4.2G	42	NA	47	12.7	143.3	5 Whole
1.0G	42	NA	47	12.8	143.3	5 Whole
OC	42	NA	47	12.5	143.3	5 Whole

Year 3 Total RNA Samples are Slightly Degraded



NASA 2002 St. 28 Total RNA Extraction

NASA 2002 St. 42 Total RNA Extraction

Preliminary Results: Year 2 and Year 3 RNA Isolation

- Year 2
 - RNA isolations have been successful
 - Visual inspection reveals RNA integrity is good
 - Proceed with 2.0g subtractive library construction
- Year 3
 - All RNA samples have been isolated
 - RNA appears to be degraded in St. 28 samples
 - St. 28 library construction is questionable
 - Paraffin embedded sample RNA extractions??
 - Ear RNA isolations did not yield enough RNA to be visualized
 - Proceed with ear cDNA library construction

Conclusion

- Preliminary results show *X. laevis* development is affected little, if at all, by chronic acceleration
- I have optimized RNA isolating techniques and I'm currently practicing cDNA library construction techniques
- RNA samples have been isolated from year 2 and year 3
- Subtractive cDNA library construction methods are being scrutinized

What I expect to achieve

- Random screens of the library will be performed to evaluate differential gene expression in chronic acceleration
- *X. laevis* hypergravity subtractive cDNA libraries can be used for construction of gene chips for microarray analysis in future hypergravity studies

Acknowledgements

- Dr. Elba E. Serrano
- Members of the Serrano Laboratory
- Members of the NASA AMES Research Facility
- Funding provided by NMSGC Graduate Fellowship (2002/2003)
- Supported by a NASA MURED FAR award to EES

