



MIGRATION

- Large mammals
- Birds
- Monarch Butterflies

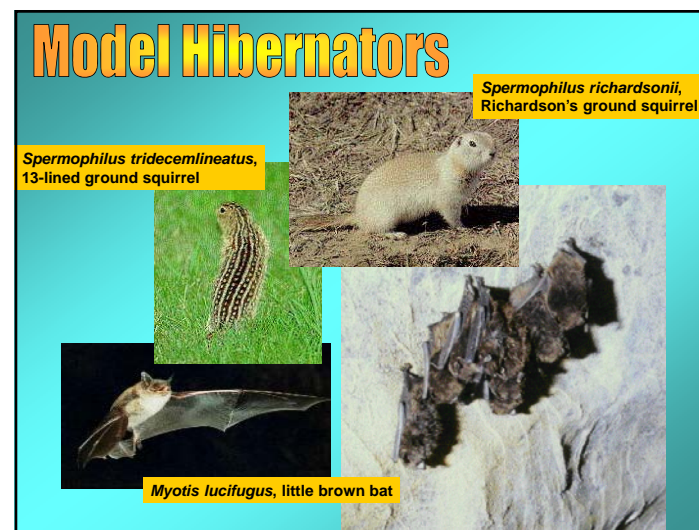
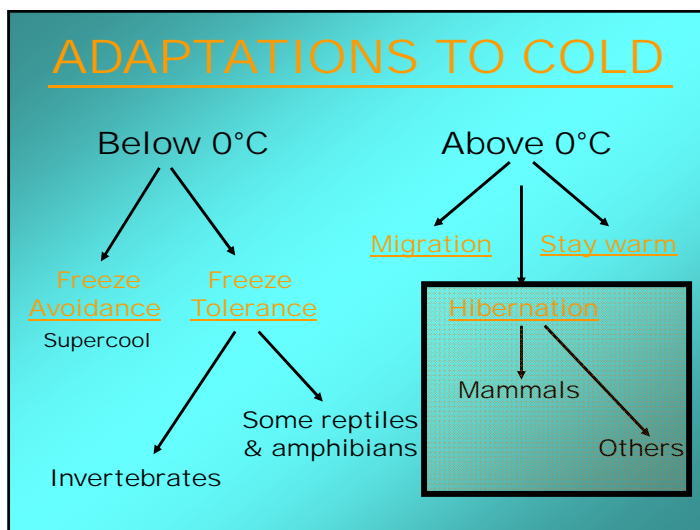
Species able to book airline flights

Latitudinal & Altitudinal

A photograph of a beach scene. The foreground is a sandy beach with some footprints. In the background, there are mountains and a cloudy sky. The image is set against a light blue gradient background.

Migrating DOWN


A photograph of a frog with a mottled pattern, sitting on a rock. The frog is looking towards the right. The image is set against a light blue gradient background.A photograph of a pile of earthworms. The worms are pinkish-brown and are coiled together. The image is set against a light blue gradient background.





- Seasonal phenomenon
- Pre-hibernation hyperphagia
- Gain up to 40% of body mass
- Need polyunsaturated fats
- Find hibernaculum: dark, near 0°C

CELL PROCESSES

- 
- DNA/RNA synthesis
 - Protein synthesis
 - Fuel metabolism
 - Ion pumping
 - Work done

ATP turnover ↓ to <5% of normal

METABOLIC RATE DEPRESSION

1. Slow Cell Processes
2. Use protein kinases (activate SAPKs)
3. Selective gene activation

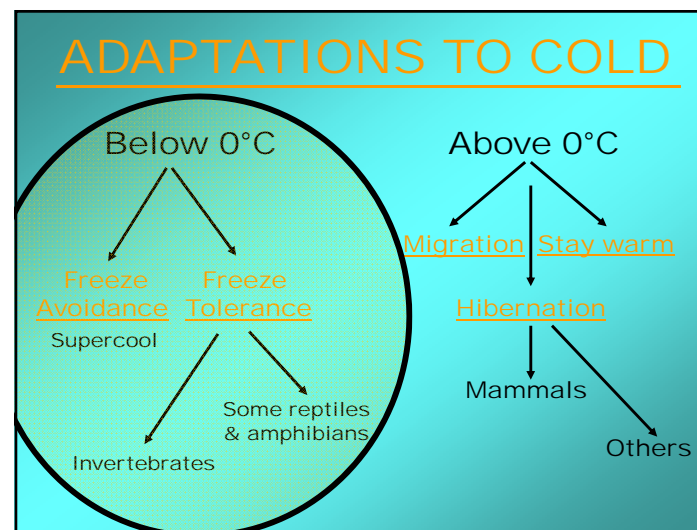
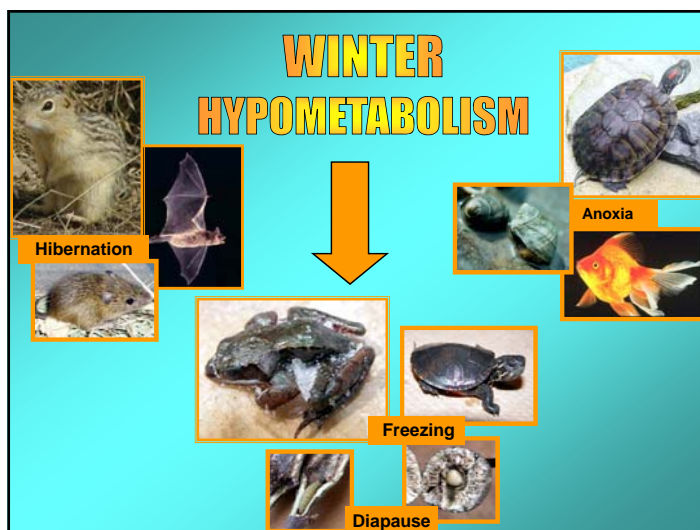
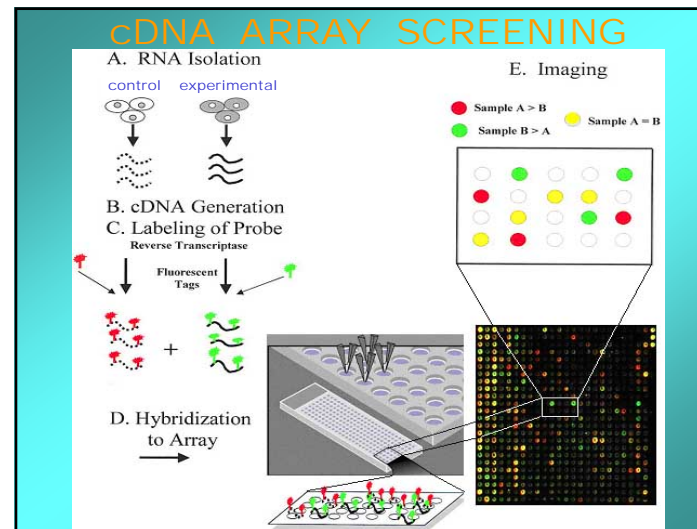
METABOLIC RATE DEPRESSION

- Protein Synthesis slows to 1%
- Pumps & Channels closed
- Energy Production slows to 5%
- Energy Utilization slows to 2%
- Few 'SAP' kinases activated
- Gene 'inactivation'
- Few Genes activated

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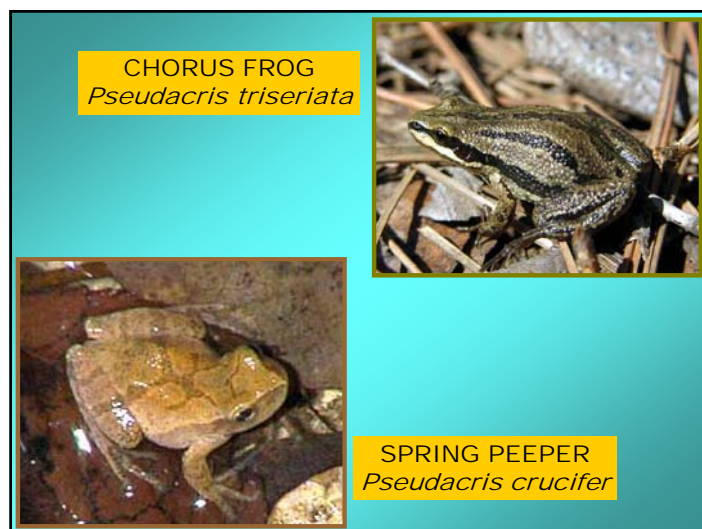
- Gene 'inactivation' (miRNA)
- Few Genes activated (1 % only)



FREEZE TOLERANT ANIMALS

- TERRESTRIAL INSECTS
- INTERTIDAL MOLLUSCS & BARNACLES
- AMPHIBIANS & REPTILES:
 - FROGS (6 species)
 - HATCHLING PAINTED TURTLES
 - GARTER SNAKES
 - LIZARDS (some)







WOOD FROG
Rana sylvatica

A WOOD FROG LIFE

- SUMMER** - spent in the woods, eating & growing
 - AUTUMN** - hide in insulated spots on forest floor
 - WINTER** - freeze when hibernation site falls to about -2°C; survive frozen to -10°C
 - SPRING** - thaw & revive, move to woodland ponds
- Mating & egg laying** - within 1 week in early spring
- Eggs & tadpoles** - develop fast before temporary ponds dry out; metamorphosis in early summer

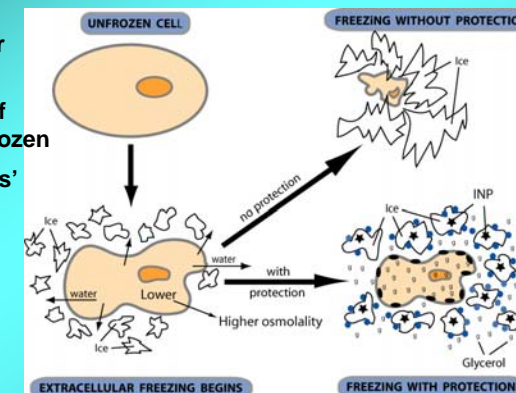
Frogs of various colours are numerous in those parts as far North as the latitude 61°...as the Winter approaches, they burrow under the moss, at a considerable distance from the water, where they remain in a frozen state till the Spring. I have frequently seen them dug up with the moss (when pitching tents in Winter) frozen as hard as ice; in which state the legs are as easily broken off as a pipe-stem, without giving the least sensation to the animal; but by wrapping them up in warm skins, and exposing them to a slow fire, they soon recover life...". Samuel Hearne

A Journey from Prince of Wales's fort in Hudson's Bay to the Northern Ocean in the Years 1769-1772



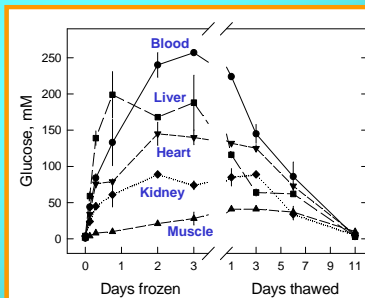
SURVIVING FREEZING

- Extracellular freezing only
- Up to 70% of body water frozen
- High 'polyols'
- Acclimation required
- Glucose
- Glycerol
- Sorbitol



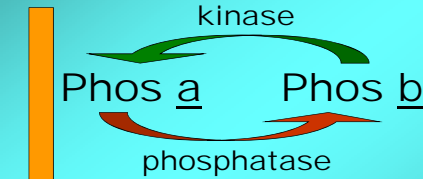
WOOD FROG CRYOPROTECTANTS

- Blood glucose rises from ~5 mM to 200-400 mM
- Glucose triggered by ice formation
- Made from liver glycogen (180 mg/g)
- Liver is ~12% of body mass
- Glucose distribution via Blood:
Liver >
Core organs >
Periphery

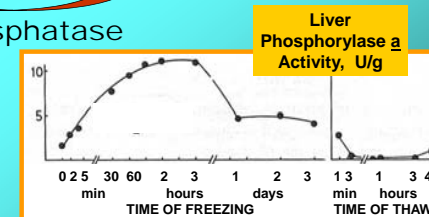


GLYCOGEN PHOSPHORYLASE

Glycogen + P_i



Glucose-1-P + glycogen (n-1)



TO SURVIVE FREEZING

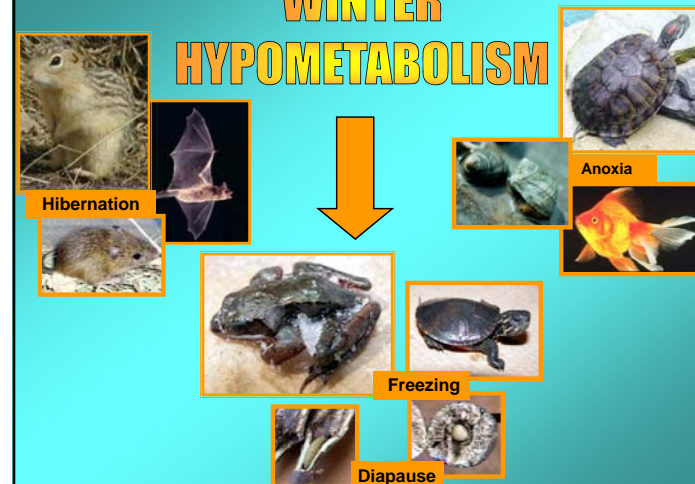
- Alter metabolism to synthesize cryoprotectants (polyols, sugars)
- Defend against intracellular desiccation
- **Suppress metabolic rate**

ACCOMPLISHED BY:

- Activate signaling enzymes in every cell
 - 'SAP' kinases
 - Role: reversible controls on cell processes

★ Up-regulate selected genes

WINTER HYPOMETABOLISM



FREEZE INDUCED CHANGES

- Gene 'inactivation'
- Protein Synthesis slows to 1%
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FREEZE-INDUCED GENES: WOOD FROGS

cDNA Library / Gene Chip

- Only 1 % of genes "on"
- The Unknowns:
Fr10, Li16, FR47

Storey KB 2004. Strategies for exploration of freeze responsive gene expression: advances in vertebrate freeze tolerance. Cryobiology 48, 134-145

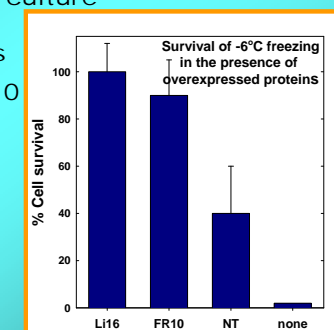
THE UNKNOWNNS : LI16, FR10, FR47

- Novel gene sequences discovered by cDNA library screening
- Genes moved to other cell types
- Genomic sequences now known
- On-Off Regulation: Protein Kinases
- Proteins are biomanufactured in our lab
- Non freeze tolerant cells can be transformed



FUNCTION OF THE UNKNOWN PROTEINS

- Express genes in cells in culture
 - Li16, FR10
 - insect or mammal cells
- Expression of Li16 & FR10 protects cultured cells from freezing damage
- Li16 is intracellular
- FR10 is exported
- Both bind to membranes



Unique Animal Stress Model



Vertebrate
whole-body
freeze tolerance

Tissue
cryopreservation



Tolerance of extreme
ischemia and
hyperglycemia

CRYOPRESERVED TISSUES

- SPERM
- EMBRYOS
- SKIN
- CORNEA
- VEINS
- BLOOD CELLS
- HEART VALVES
- TEETH, BONE
- BONE MARROW
- PANCREATIC TISSUE
- THYROID TISSUE
- PARATHYROID TISSUE
- FETAL TISSUES (some)
- ****RAT LIVER****

ORGANS FOR TRANSPLANT

1. Scientific Solutions

A. IMMEDIATE: extend the viability of removed organs by hours/days

B. FUTURE:

- freeze organs to create organ banks
- stem cell research - grow new organs

C. FAR FUTURE:

- cloning of tissues (one cell --> organ)
- artificial tissues (from non-cell sources)

D. XENOTRANSPLANTS

- Dangers and risks?

E. Clone "NEAR-HUMANS" for parts:

- Society plus science (+/- embryos)
- Have your own clone, just in case?
- The rights of a clone?

ORGANS FOR TRANSPLANT

Tens of thousands wait for a few organs

- who decides?
- should you be able to pay for an organ?

2. Society Solutions: Dollars, Science, Morals

A. SELL ORGANS: \$\$ from rich to poor people
Organs from poor to rich people
Morally correct? How to regulate?

B. Get organs by "PRESUMED CONSENT"

- Will doctors revive or harvest?
- How dead do you have to be?
- Religious / spiritual implications

THE FUTURE ??



FROZEN IN TIME

A WOMAN WHO should have died in 1959 has been saved by modern heart surgery — after spending the last 50 years in a deep freeze.

Woman put in deep freeze in 1959 revived by doctors and given life-saving heart surgery

At the age of 20, Helene Chapparral had been placed in a state of suspended animation by doctors who lacked the medical know-how to repair her ill heart.

That latter-making moment came in 1959 when the population of Los Angeles received the first heart transplant using newly-developed surgical techniques.

Dr. Michael Freudenthal, 88, headed the team of doctors who brought Helene back to life. He had also been present in 1959 when she was frozen.

"I had just graduated from medical school," he recalls of that time. "I was invited to attend a lecture on suspended animation by a young doctor who was named to see the young doctor listed in an announcement in the medical journal."

Dr. Freudenthal explained that the woman suffered from heart disease and had only been frozen for a few days.

The estimated procedure called for her body to be frozen, and then revived while medical science had developed a cure for her ailment.

"When her body temperature reached the freezing point, her blood was removed and her veins were washed with a glycerol-water solution. She was then placed in a coffin-like tube containing liquid nitrogen at minus 200 degrees Fahrenheit."

Helene's frozen body was stored in a hospital storage room, where it remained for the next 50 years.



HELENE CHAPARRAL spent 50 years in deep freeze



DR. FREUDENTHAL was present when Helene was frozen and after when she was brought back to life



HELENE WAS put in freezing tank similar to one above used by New Cypriot Society

Scientists revive frozen human brain!

Soviet scientists revived and held a conversation with the brain of a man who fell into an icy Siberian crevasse in 1921 and remained frozen until the discovery of the body earlier this year.

In a report to the science journal *Mind and Body*, Dr. Alexei Kolomoiedt concluded that his conversation with the brain was short but called it groundbreaking because it proved that human memory and intelligence can survive long-term freezing.

Organ linked to computer and answers stunned docs' questions

Dr. Kolomoiedt's questioning indicates that the brain was aware and able to answer his questions. "We asked such things as 'Are you comfortable?' 'Do you remember your name?' 'Do you remember your name?' 'Do you remember your name?' 'Do you remember your name?'"

These messages, which were received from the brain and linked to a computer grid by an electrical connection with it. If there answered you no to sit simple questions before it began to come and shut down.

"We did not raise the dead but we came very, close," said the scientist.

First a few minutes the brain waves were nearly as active as those of a living man.

Heaven Can Wait a While

A woman loses her head

She spent 50 years after death but, miraculously, her head still was preserved. Helene Chapparral, who had lived with her heart of an 18-year-old girl, died with her heart in suspended animation. After cut off her mother's head and froze it in liquid nitrogen, against the odds, Dr. Michael Freudenthal, 88, headed the team of doctors who brought Helene back to life.



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After several hours, Helene was brought back to life.

Coroner: Woman's death murder; head then frozen

A 20-year-old woman whose head was surgically removed and frozen in hope that she would come back to life with a new body.

"We're trying to save her as a 20-year-old body that was 82 and pushed over the edge by the use of a drug," says Deputy Coroner Carl Hansen.

Deputy District Attorney Carl Hansen said he is "fairly confident" that the woman's death was a homicide.

The woman's head, which was surgically removed and frozen in hope that she would come back to life with a new body.

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FREEZING HUMANS

DOES IT WORK ?

- A. Liquid Nitrogen Storage (-196°C)
 - frogs only to -20°C (cell destruction)
 - fragility/crush (neurons)
- B. Frozen Liquid Expands !
- C. Bits and Bobs
- D. Time to Preservation (oxygen lack, neurons)
- E. You've paid UPFRONT for "forever" !
- F. Legal implications (thawed by your kids)

FREEZING HUMANS

Is it correct to freeze humans and then bring them back in the future for "eternal life"?

- A. Who would be chosen for this (costly) procedure?
- B. How would we pay for re-animation and re-integration into society?
 - for 20 subjects
 - for 2000 subjects
 - for 2 billion subjects
- C. Spiritual / Religious implications
- D. Legal implications

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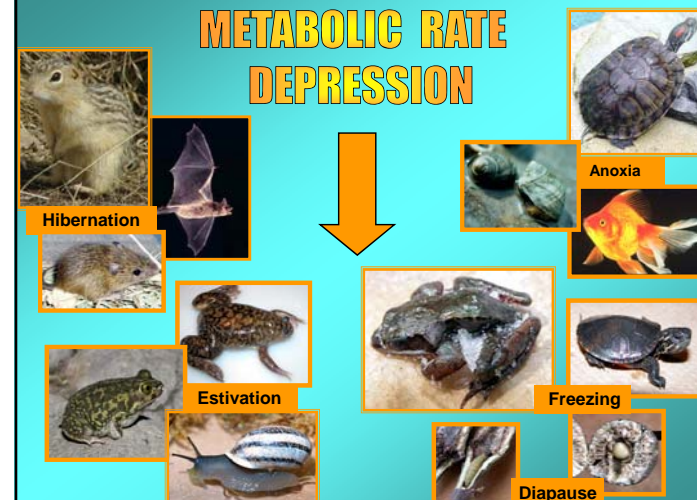
THE LAB

Research interests	Evolution of hibernation
Positions available	NEW Positions & Postdoc. Fellowships
Lab personnel, Past and Present	RECENT Publications 1996 - Present
Essa computer assistance	Publications 1986 - 1995
Array II: Inks on running cDNA arrays	Publications 1974 - 1985
Photo gallery...our animals & their stories	

BOOKS

Cell and Molecular Responses to Stress Elsevier Science	Functional Mechanisms of Cells John Wiley & Sons
Environmental Stress and Gene Regulation BOS Scientific Publishers, 1999	Molecular Mechanisms of Metabolic Arrest BOS Scientific Publishers, 2001

METABOLIC RATE DEPRESSION



FREEZE TOLERANCE

- J. STOREY
- D. McNALLY
- J. MacDONALD
- T. CHURCHILL
- S. GREENWAY
- C. HOLDEN
- S. WU
- A. DeCROOS
- L. ZHENHONG
- J. DU
- Q. CAI
- F. SCHUELER
- S. BROOKS
- B. RUBINSKY
- R. BROOKS

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