LIVING WITHOUT OXYGEN

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LITTORINA LITTOREA

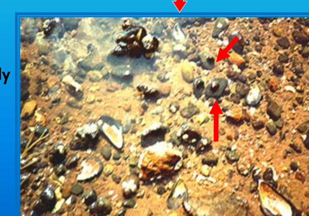
- Marine gastropod (periwinkle)
- Found on the Atlantic coast
- Number of species decreases drastically from south to north.
- Intertidal zone highly variable environment

INTERTIDAL ZONE

Ingonish Highlands, Cape Breton Island (Nova Scotia, Canada)

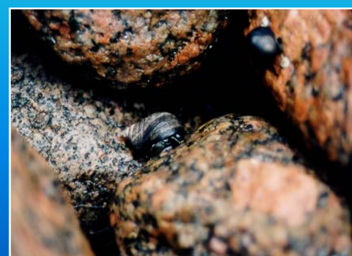
Incredibly buff research scientist

INTERTIDAL POOL



- Highly variable : temperature, salinity, oxygen, pH
- Temperature & oxygen fluctuate daily, seasonally and from pool to pool
- At low tide gill-breathers exposed to air causing hypoxia and/or anoxia
- At low tide in winter air temp >> colder than water = tissue freezing

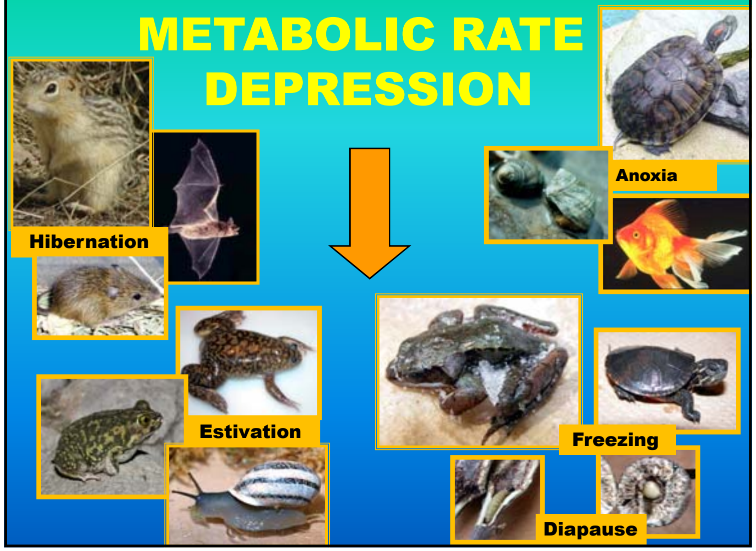
Inside the Tide Pool



A temporary environment is created during every LOW TIDE.

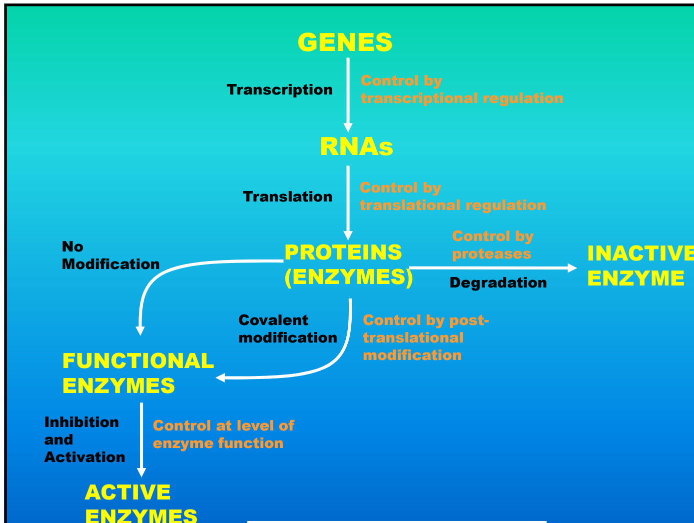
Air exposure ⟶ Hypoxia

METABOLIC RATE DEPRESSION



QUANTIFYING METABOLIC RATE DEPRESSION

- Oxygen consumption
- Calorimetry
- ATP turnover rate
- Cross-over studies on pathway flux
- Enzyme kinetic /-P analysis
- Gene Regulation



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No Modification

PROTEINS (ENZYMES)

Control by proteases

Degradation

INACTIVE ENZYME

Covalent modification

Control by post-translational modification

FUNCTIONAL ENZYMES

Inhibition and Activation

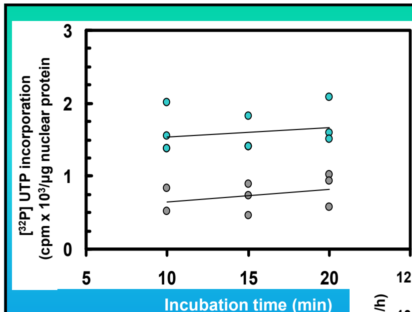
Control at level of enzyme function

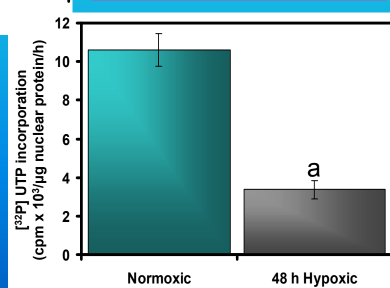
ACTIVE ENZYMES

METABOLISM IN ANOXIA

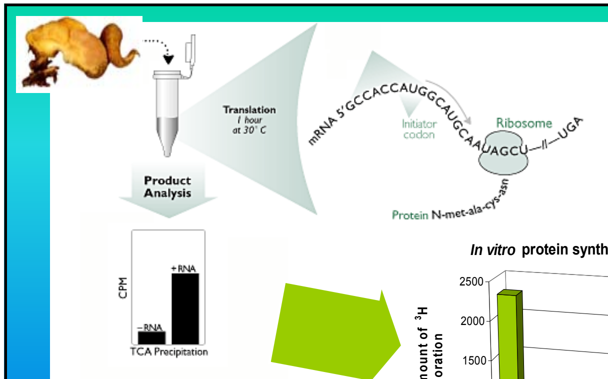
- **mRNA synthesis**
- **Protein synthesis**
- **Fuel use (incl. CHO)**
- **O_2 consumed**

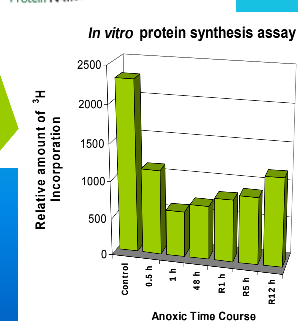
ATP turnover ↓ to <5% of normal



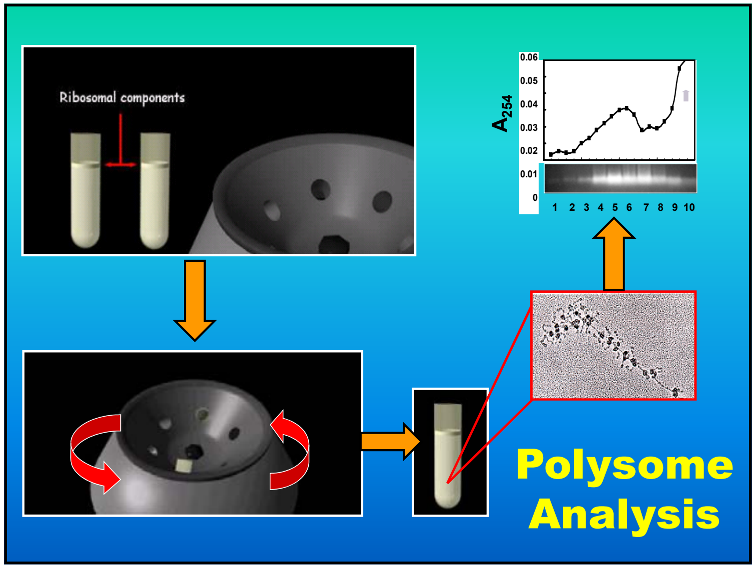


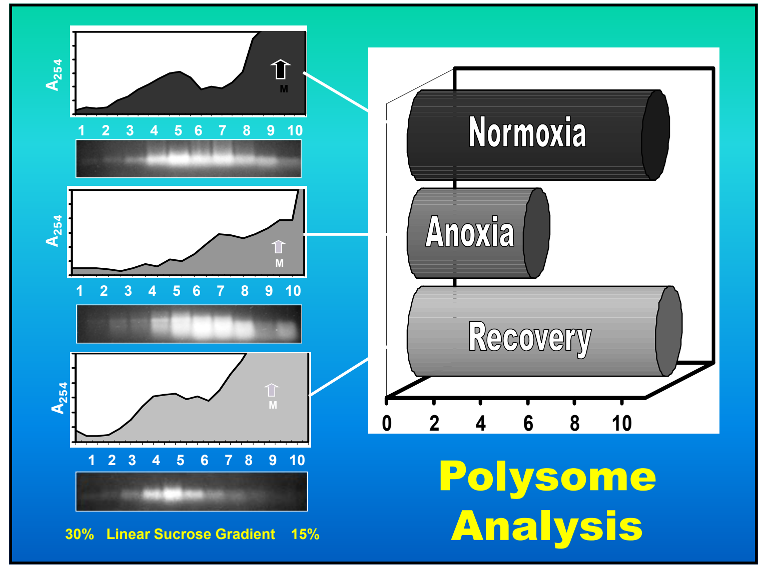
mRNA synthesis ↓

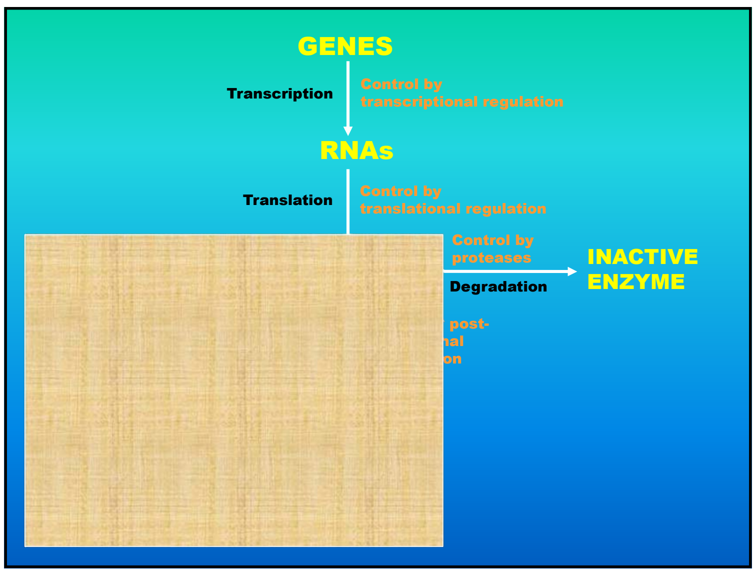




***In vitro* Protein Synthesis**







PRINCIPLES OF METABOLIC ARREST

1. **Metabolic rate reduction**
2. **Control by protein kinases**
   (SAPKs, 2nd messenger PKs)
3. **Selective gene activation**

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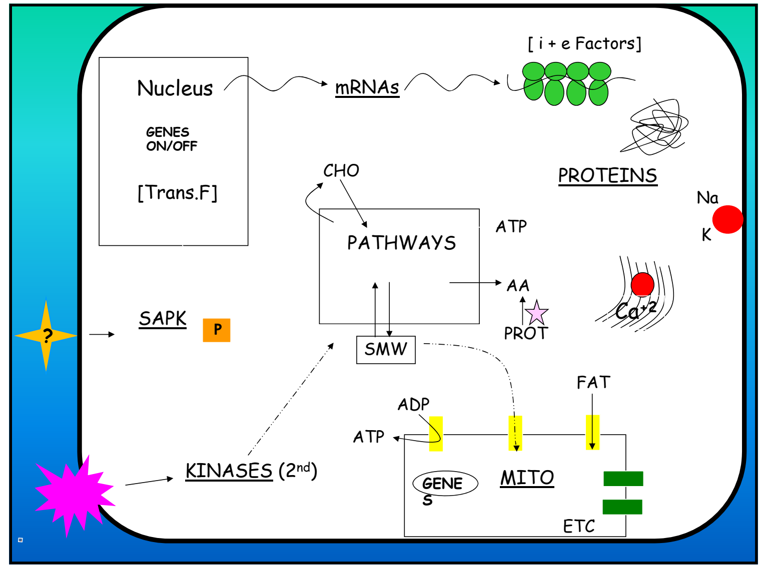
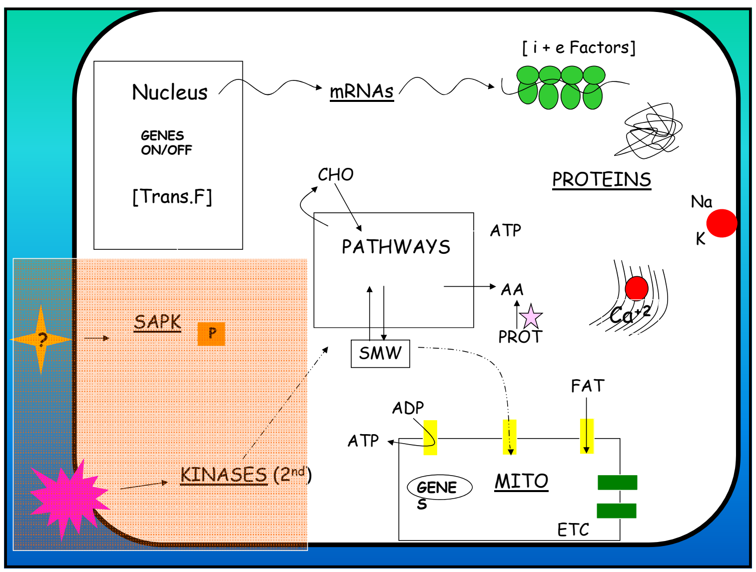
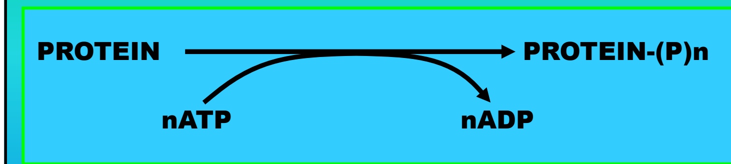




ANOXIA INDUCED CHANGES

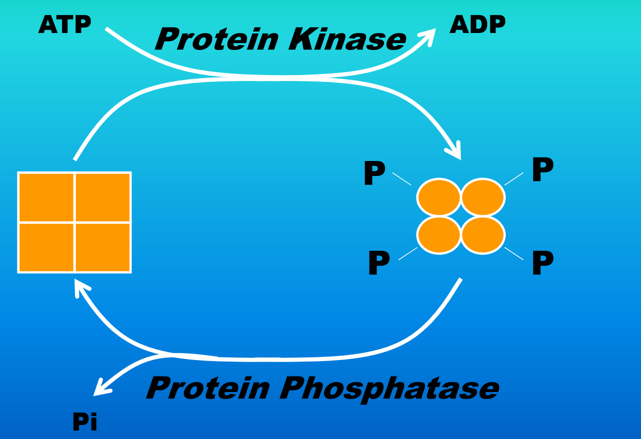
- Protein Synthesis slows to 1%
- Pumps & channels closed
- Energy Production slows to 5%
- Energy Utilization slows to 2%
- Few 'SAP' kinases activated
- Gene 'inactivation' (↓ mRNA)
- Few Genes activated

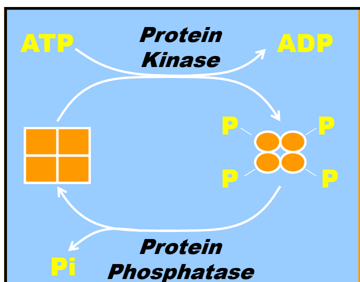
PROTEIN KINASES



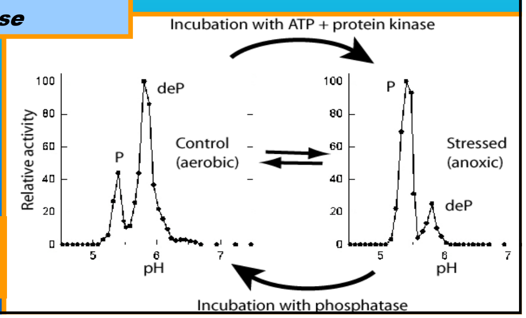
- **Covalent modification by phosphorylation**
- **Families of protein kinases: PKA (cAMP), PKG (cGMP), CaMK (Ca^{2+}), PKC (Ca^{2+}, PL,DG)**
- **SAPKs: daisy chain phosphorylations**
- **Regulation via interconversion of active vs subactive forms of protein substrates**

ENZYME CONTROL BY REVERSIBLE PHOSPHORYLATION

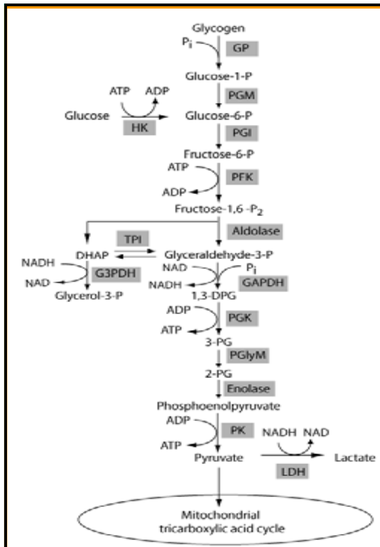




Reversible phosphorylation control of enzymes



P & deP enzymes separate on ion exchange columns

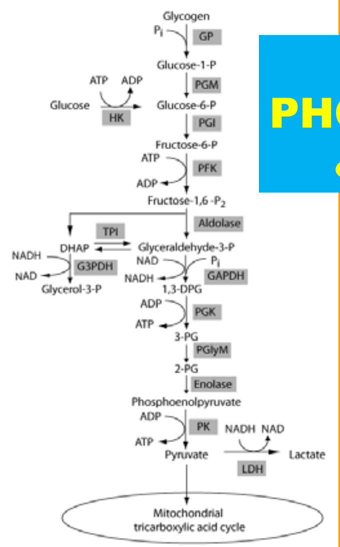


Energy production in low oxygen:

Glycolysis

1. Pasteur effect
↑ Lactate Rate

2. Metabolic depression
↓ End product Rate *alt*



REVERSIBLE PHOSPHORYLATION & GLYCOLYSIS

	P effect
GP	+ (-)
HK	-
G6PDH	-
PFK-1	-
PFK-2	-
PK	-

PROTEIN PHOSPHORYLATION & GLYCOLYSIS

- Protein kinase A , cAMP
- PKG , cGMP
- Protein kinase C
- Protein phosphatase 1, 2A, 2C

PATHWAY CONTROL IN MR DEPRESSION

Phospho / de-Phospho

- Glycolysis (GP, GS, PFK, PK)
- Fat synthesis (ATP-CL, ACC)
- CHO fuel use (PDH)
- Translation (eIF2α, eEF2)
- Ion pumps (NaK-ATPase, Ca-ATPase)

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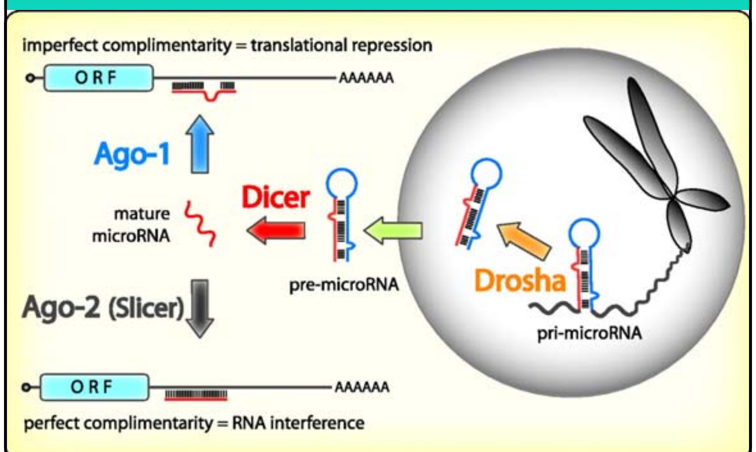
TURNING OFF GENES: Role of Epigenetics

Epigenetics:

stable changes in gene activity that do not involve changes in DNA sequence.

Common mechanisms:

- DNA methylation
- Histone modification/histone variants
- Regulatory non-coding RNAs
Hibernators! Frogs! Littorines ?



Cuellar TL, McManus MT. J Endocrinol. 187(3):327-332, 2005.

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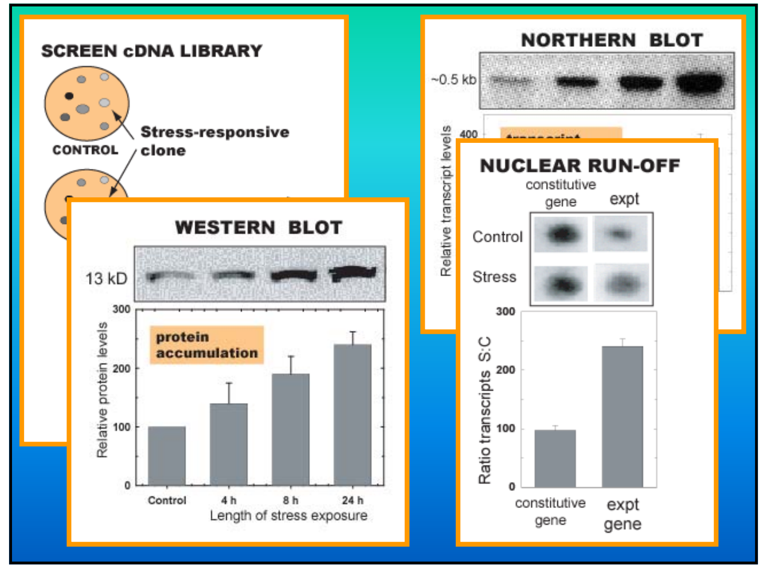
ROLE OF TRANSCRIPTION

- Global rate of mRNA synthesis depressed. Method: nuclear run-on
- Are selected genes up-regulated ?
- TO ASSESS GENE UPREGULATION:

What new mRNAs are created
- cDNA library, Gene Chip



GENE CHANGES IN Anoxic Littorina

- AOE
- Mitochondrial Genes
- Transporters
- Shock proteins (GRP, HSP)
- Low oxygen Shock (HIF)
- Transcription factors

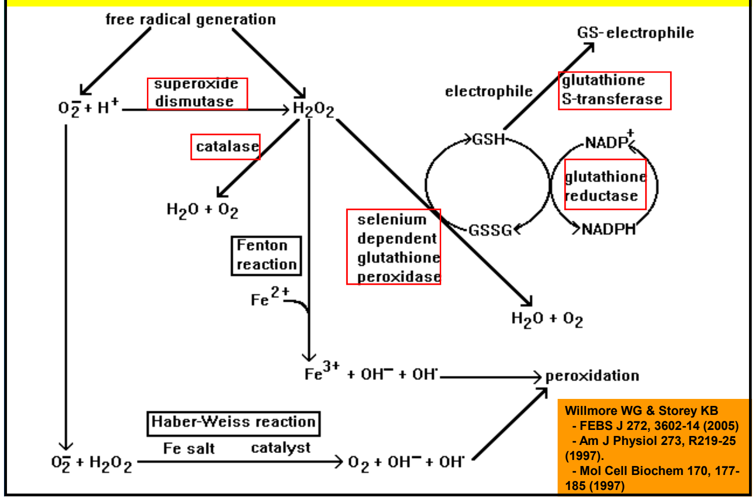
• DNA Chip ~1-2% ⇧

GENE CHANGES IN Anoxic Littorina

- Antioxidant Enzymes
- Mitochondrial Genes
- Transporters
- Shock proteins (GRP, HSP)
- Low oxygen Shock (HIF)
- Transcription factors

• DNA Chip ~1-2% ⇧

ANTIOXIDANT ENZYMES



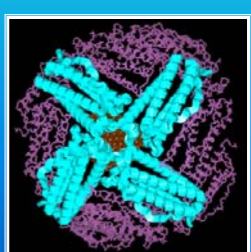
Willmore WG & Storey KB
- FEBS J 272, 3602-14 (2005)
- Am J Physiol 273, R219-25 (1997).
- Mol Cell Biochem 170, 177-185 (1997)

ANTIOXIDANT DEFENSE

- **Iron storage:**
  - **- Ferritin (H & L chains)**
  - **- Transferrin receptor 2**
- **Antioxidant enzymes**
  - **- SOD (1)**
  - **- GST (M5, A2)**
  - **- GPX (1, 4)**
  - **- Peroxiredoxin 1**

Ferritin heavy chain

- Sequesters and stores iron
- Protein shell holds up to 4,500 atoms of iron
- Shell has 24 subunits in combining : light (19 kDa) and heavy (21 kDa) subunits
- Iron sequestering reduces oxygen free radical production via the **FENTON REACTION**



$O_2 + e^- \rightarrow O_2^{\cdot-}$ superoxide radical

$O_2^{\cdot-} + H_2O \rightarrow HOO^{\cdot} + OH^-$ hydroperoxyl radical

$HOO^{\cdot} + e^- + H^+ \rightarrow H_2O_2$ hydrogen peroxide

$H_2O_2 + e^- \rightarrow {}^{\cdot}OH + OH^-$ hydroxyl radical

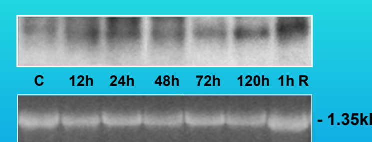
70's radicals

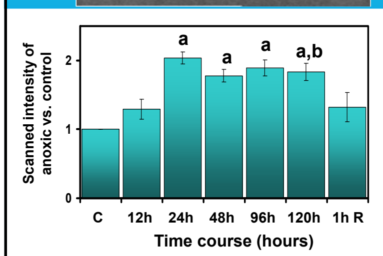
$Fe(III) + O_2^{\cdot-} \rightarrow Fe(II) + O_2$

$Fe(II) + H_2O_2 \rightarrow Fe(III) + {}^{\cdot}OH + OH^-$ Fenton reaction

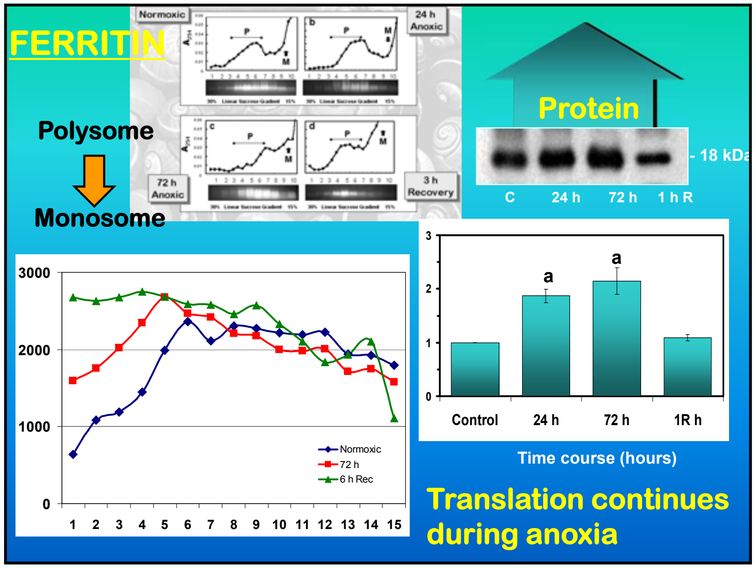
$O_2^{\cdot-} + H_2O_2 \rightarrow O_2 + {}^{\cdot}OH + OH^-$ Haber-Weiss reaction

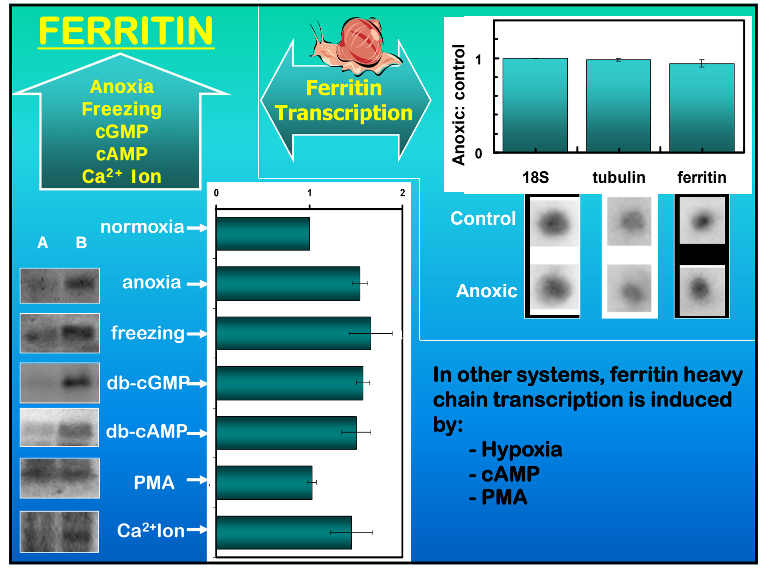
Ferritin
heavy chain

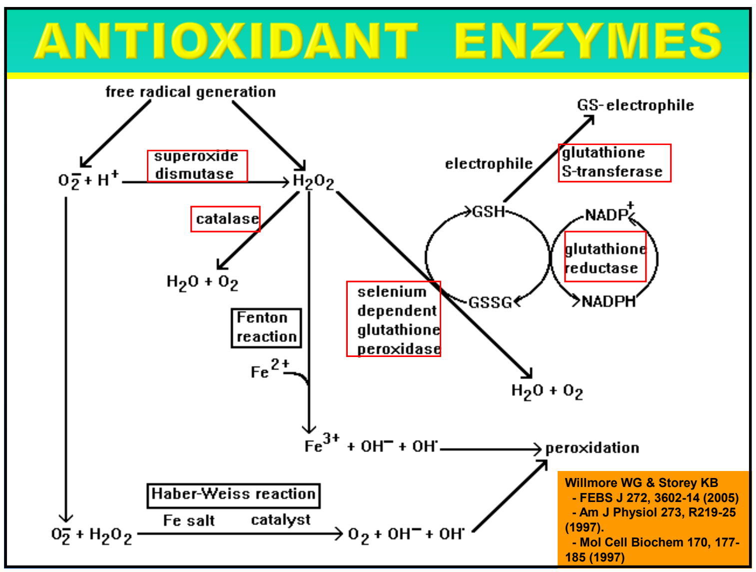





```
littorina   ........................IKELG
otala       LCTGHEDAQMADFLETEFLEEQVKSIKEIA
lymnaea     LCTSHDDAQMADFLESEFLEEQVKSIKELS
salmon      VCSEHNDPHMCDFIETHYLDEQVKSIKELG
Consensus                           ike
littorina   DHITNLKRVGSGL...GEYEYDKNLSS
otala       DHITNLKRVGSGL...GEYVYDKESLDE
lymnaea     DYITNLKRVGPGL...GEYIFDKETLSSSS
salmon      DWVTNLRRMGAPQNGMAEYLFDKHTLGKES
Consensus   d  tnl r g       ey  dk
```



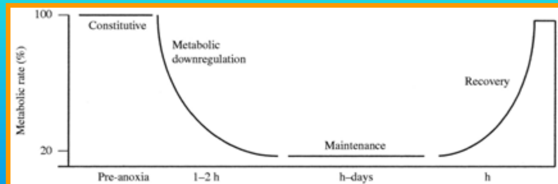




PRINCIPLES OF HYPOXIA SURVIVAL

1. Metabolic rate depression
2. Alternative end products
3. Reversible phosphorylation of enzymes
4. Overall suppression of transcription & translation
5. Selected genes up-regulated

ANOXIA SURVIVAL



Constitutive factors	Metabolic downregulation	Basal maintenance	Recovery
Glycogen	Hypoxia/energy failure sensing	ATP turnover rates minimal	Oxygen sensing
Opioid receptors	HIF-1α ↑, glycolysis ↑	Preserve network integrity	Ion channels ↑
GABA receptors	ATP ↓↑, adenosine ↑	Ion channel ↔	Electrical activity ↑
Hsp72, Hsc73	Ion channels ↓, electrical activity ↓	Electrical activity ↔	Protein synthesis ↑
NF-κB	Glutamate, dopamine release ↓	Glutamate release ↔	
PACAP 38	Protein synthesis ↓	Dopamine release ↔	ROS defenses ↑
Ascorbate	Hsp72 ↑, Hsc73 ↑	GABA release ↑	
SOD		GABA receptors ↑	
	Suppression of ATP turnover rates	Protein synthesis ↔	
		Hsp72 ↓, Hsc73 ↑	

Lutz PL & Milton SL. 2004. J Exp Biol 207: 3141-3147

GENES

Transcription — Control by transcriptional regulation

RNAs

Translation — Control by translational regulation

PROTEINS (ENZYMES)

Control by proteases — Degradation → INACTIVE ENZYME

No Modification

Covalent modification — Control by post-translational modification

FUNCTIONAL ENZYMES

Inhibition and Activation — Control at level of enzyme function

ACTIVE ENZYMES

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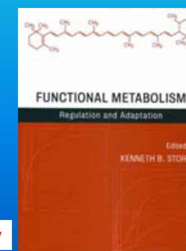

ANOXIA

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2517

Freezing and anoxia stresses induce expression of metallothionein in the foot muscle and hepatopancreas of the marine gastropod *Littorina littorea*

Tamara E. English* and Kenneth B. Storey†

Institute of Biochemistry and Department of Biology, College of Natural Sciences, Carleton University, 1125 Colonel By Drive, Ottawa Ontario, Canada K1S 5B6

*Present address: HHMI at the Mount Sinai School of Medicine, Department of Physiology and Biophysics, New York, NY 10029, USA

†Author for correspondence (e-mail: kenneth_storey@carleton.ca)

Accepted 22 April 2002

Summary

Differential screening of cDNA libraries constructed from the foot muscle of marine snails *Littorina littorea* revealed several cDNAs that are upregulated during anoxia or freezing exposures, environmental stresses that are naturally endured by this species. One full-length clone of 1196 nucleotides (GenBank accession number AY034179) hybridized with a 1200-nucleotide band on northern blots and encoded a 100-amino-acid protein that was identified as belonging to the metallothionein (MT) family. *L. littorea* MT shared 45% and 56% identity with the copper- and cadmium-binding MT isoforms, respectively, from another gastropod, *Helix pomatia* and 43–47% identity with marine bivalve MTs. The *L. littorea* sequence included the mollusc-specific C-terminal motif Cys-X-Cys-X(3)-Cys-Thr-Gly-X(3)-Cys-X-Cys-X(3)-Cys-X-Cys-Lys that identifies it as a family 2 (mollusc) MT. Northern blot analysis showed that *L. littorea* MT was upregulated in both foot muscle and hepatopancreas in response to both freezing and anoxia stresses; within 1 h of the beginning of the stress transcript levels rose 2.5- to sixfold of control levels, reaching maximal levels at 12 or 24 h. After 24 h recovery from either stress, transcript levels were reduced again in three cases but remained elevated in hepatopancreas from anoxia-treated snails. Upregulation of MT during environmental stress could serve one or more possible roles, including a function in antioxidant defense.

Key words: environmental stress, gene expression, metallothionein, invertebrate, anaerobiosis, freeze tolerance, periwinkle, *Littorina littorea*.

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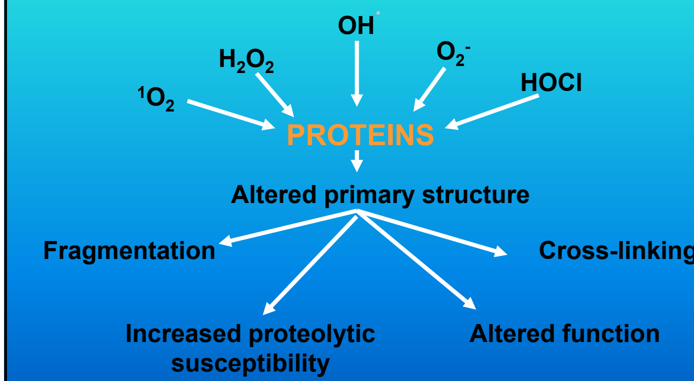
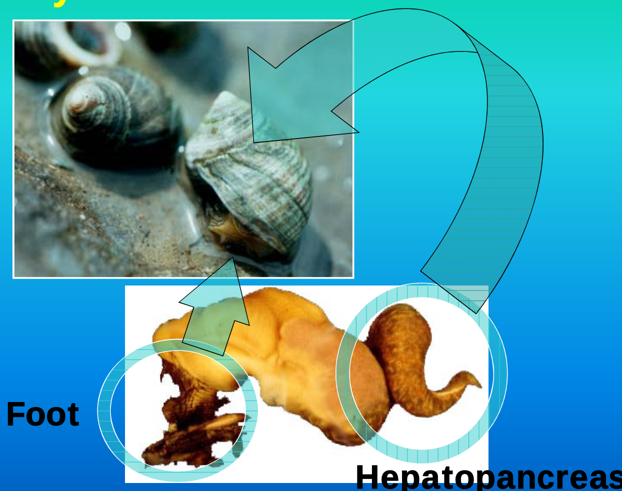
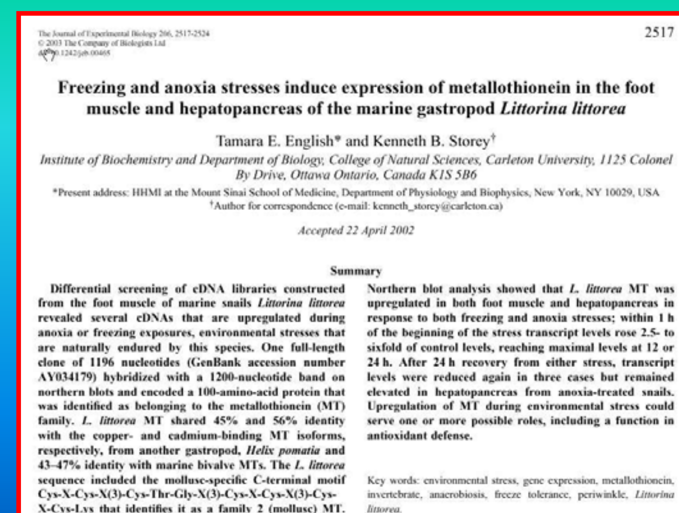
OXYRADICAL DAMAGE TO PROTEINS



Anatomy of *Littorina littorea*





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$H_2O_2 + e^- \longrightarrow {}^{\cdot}OH + OH^-$ hydroxyl radical

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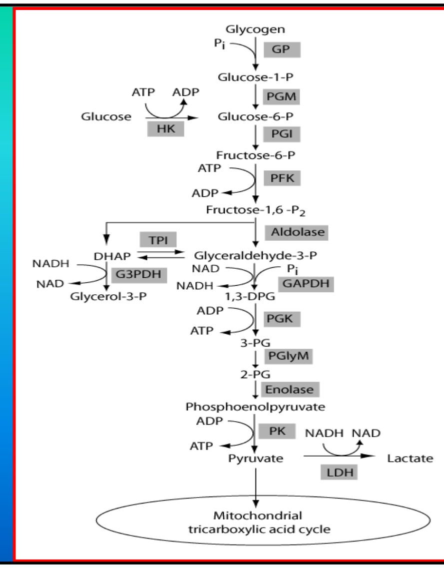
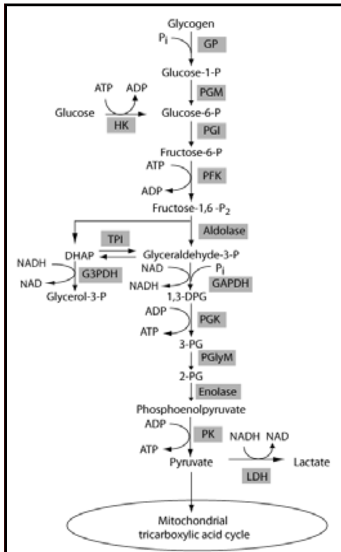
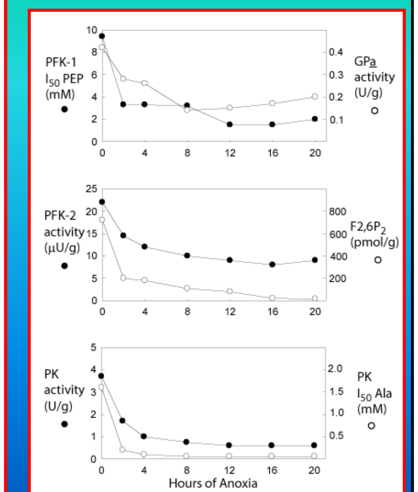
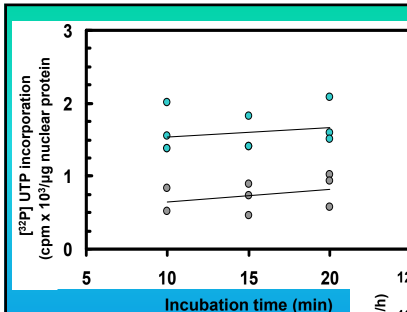
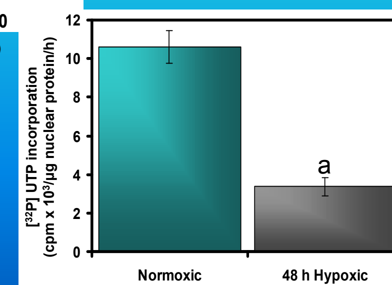


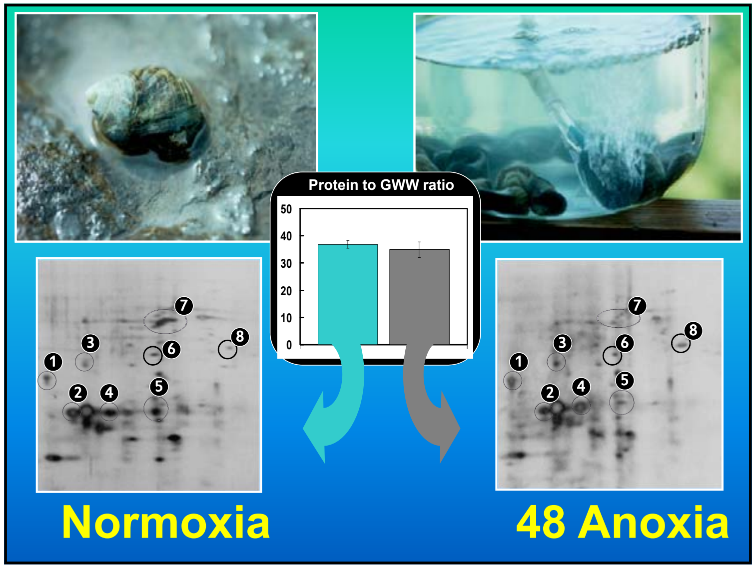


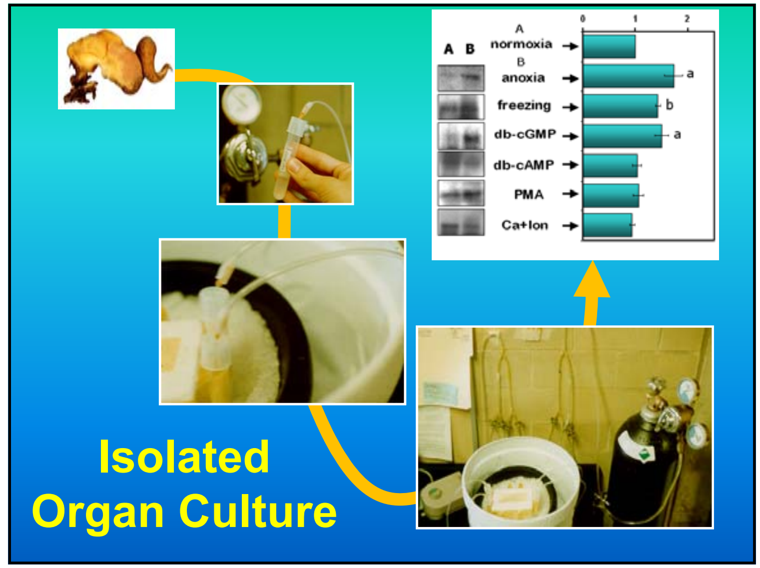




mRNA synthesis







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Reversible suppression of protein synthesis in concert with polysome disaggregation during anoxia exposure in *Littorina littorea*

Kevin Larade and Kenneth B. Storey
Department of Biology and Institute of Biochemistry, Carleton University, Ottawa, Ontario, Canada

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Abstract

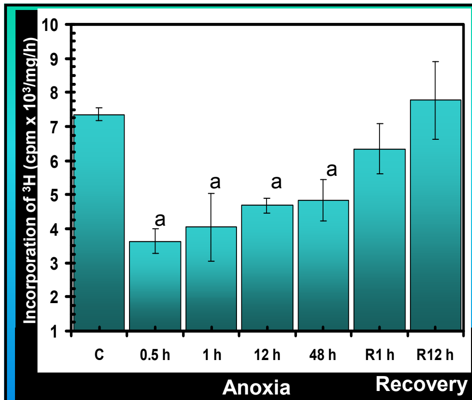
Many marine invertebrates can live without oxygen for long periods of time, a capacity that is facilitated by the ability to suppress metabolic rate in anoxia to a value that is typically less than 10% of the normal aerobic rate. The present study demonstrates that a reduction in the rate of protein synthesis is one factor in the overall anoxia-induced metabolic suppression in the marine snail, *Littorina littorea*. The rate of [^{3}H]leucine incorporation into newly translated protein in hepatopancreas isolated from 48 h anoxic snails was determined to be 49% relative to normoxic controls. However, protein concentration in hepatopancreas did not change during anoxia, suggesting a coordinated suppression of net protein turnover. Analysis of hepatopancreas samples from snails exposed to 24–72 h anoxia showed a gradual disaggregation of polysomes into monosomes. A re-aggregation of monosomes into polysomes was observed after 3 h of aerobic recovery. Analysis of fractions from the ribosome profile using radiolabeled probe to detect α-tubulin transcripts confirmed a general decrease in protein translation during anoxia exposure (transcript association with polysomes decreased) with a reversal during aerobic recovery. Western blotting of hepatopancreas samples from normoxic, 24 h anoxic, and 1 h aerobic recovered snails demonstrated that eIF-2α is substantially phosphorylated during anoxia exposure and dephosphorylated during normoxia and aerobic recovery, suggesting a decrease in translation initiation during anoxia exposure. These results suggest that metabolic suppression during anoxia exposure in *L. littorea* involves a decrease in protein translation. (Mol Cell Biochem **232**: 121–127, 2002)

PRINCIPLES OF METABOLIC ARREST

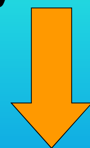
1. Metabolic rate reduction

2. Control by protein kinases
(SAPKs, 2nd messenger PKs)

3. Selective gene activation



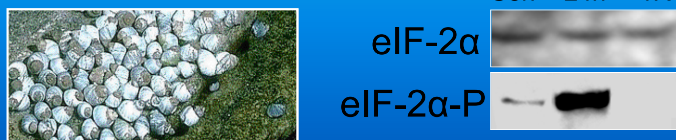
Protein Synthesis



Con 24h 1R

eIF-2α

eIF-2α-P



ANOXIA INDUCED CHANGES

- Protein Synthesis slows to 1%
- Pumps & channels closed
- Energy Production slows to 5%
- Energy Utilization slows to 2%
- Few 'SAP' kinases activated
- Gene 'inactivation' (↓ mRNA)
- Few Genes activated

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GENES

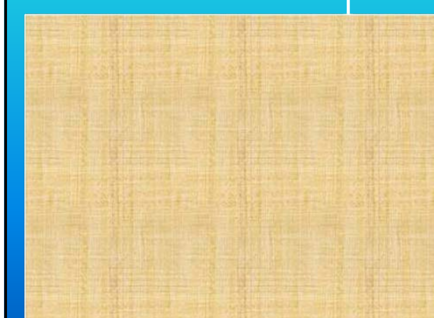
Transcription — Control by transcriptional regulation

RNAs

Translation — Control by translational regulation

Control by proteases

Degradation → **INACTIVE ENZYME**



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