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Heme Oxygenase-1: Protective Effects of Carbon Monoxide.

http://eethomp.com/bioinformatics.html

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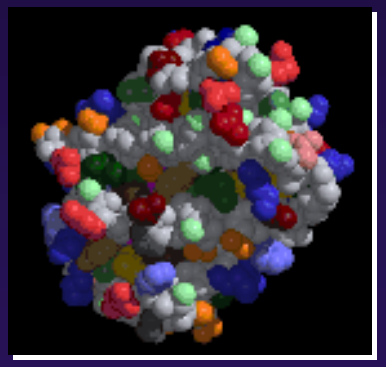

dept.physics.upenn.edu/.../hemoglobin_co.gif

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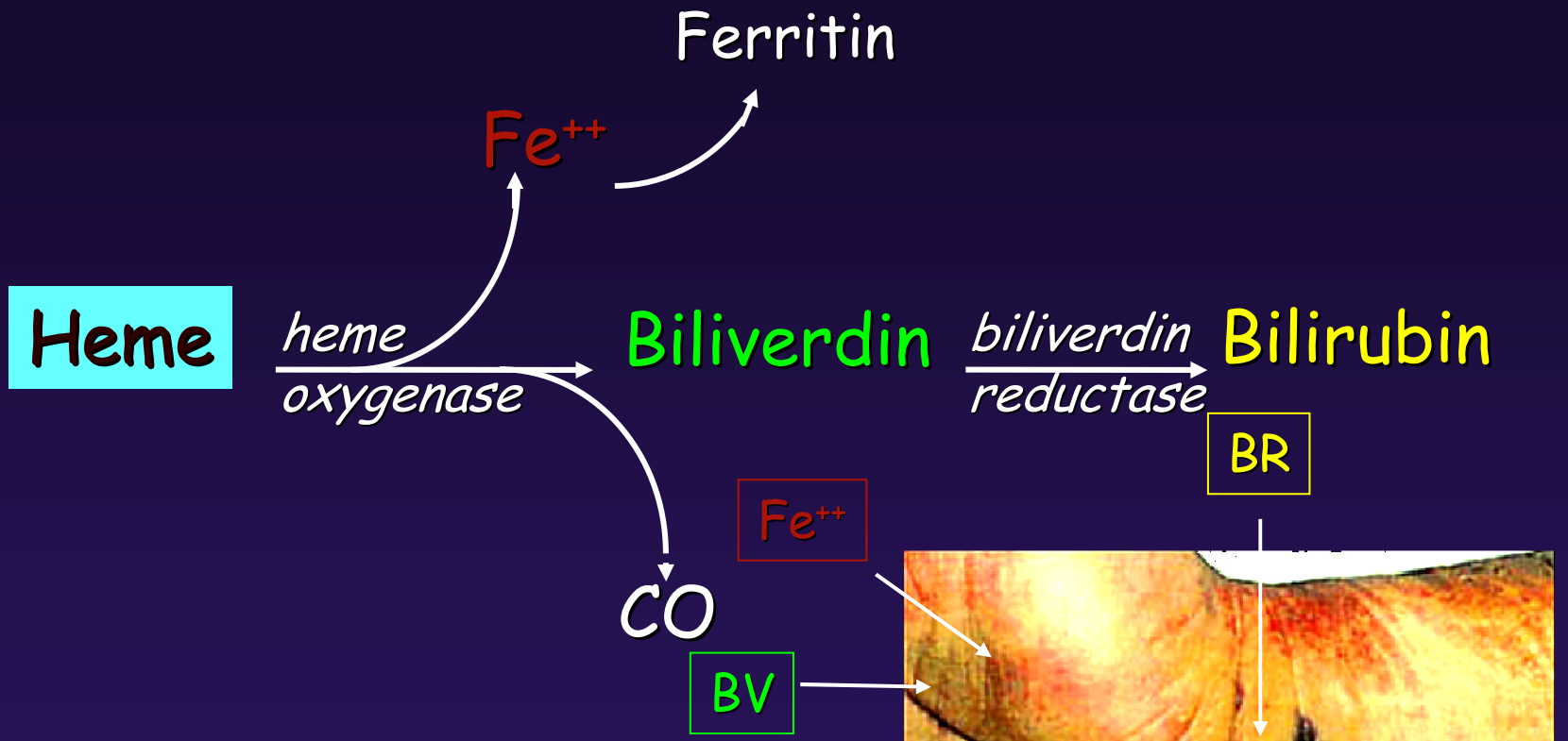
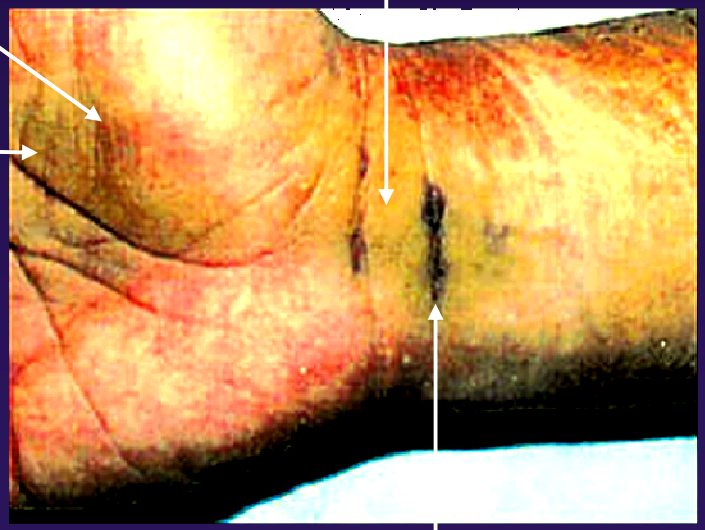
What is Heme Oxygenase-1?





http://www.aafp.org/afp/20011101/photo.html

Two isoforms of Heme oxygenase:

- *HO-1 = inducible, responsive to stress*
- *HO-2 = constitutive, brain and testes*

Heme

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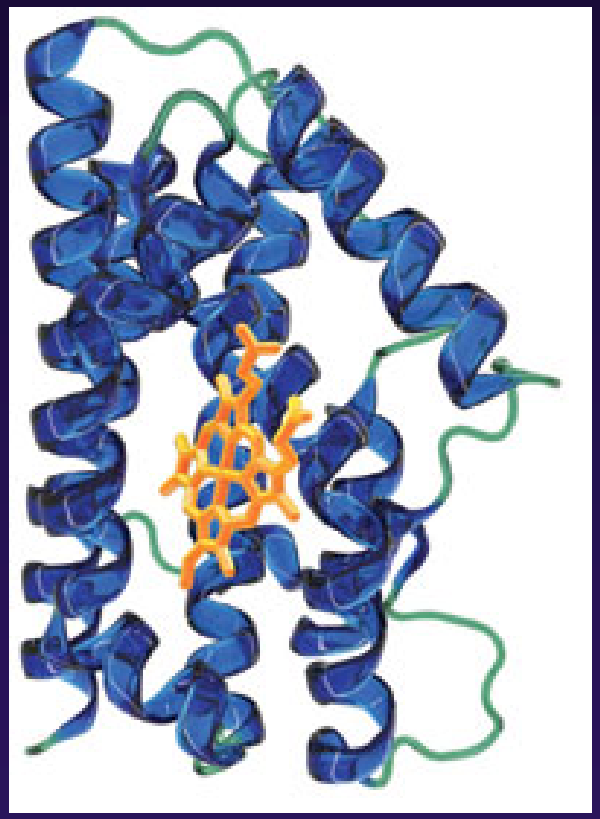
Heme Oxygenase-1 is a Stress-Response Gene



http://www.assaydesigns.com/corp/images/HO-1-structure.jpg

- H_2O_2
- Hyperoxia
- UVA
- Endotoxin
- Hypoxia
- Heavy Metals
- Cytokines
- Nitric Oxide (NO)

Why is Heme Oxygenase-1 important: Consequences of HO-1 deficiency

Poss et al, PNAS 1997.94:10925-30

Murine model

- Anemia.
- Iron deposition.
- Susceptibility to stress and injury.
- Exacerbation of myocardial injury.

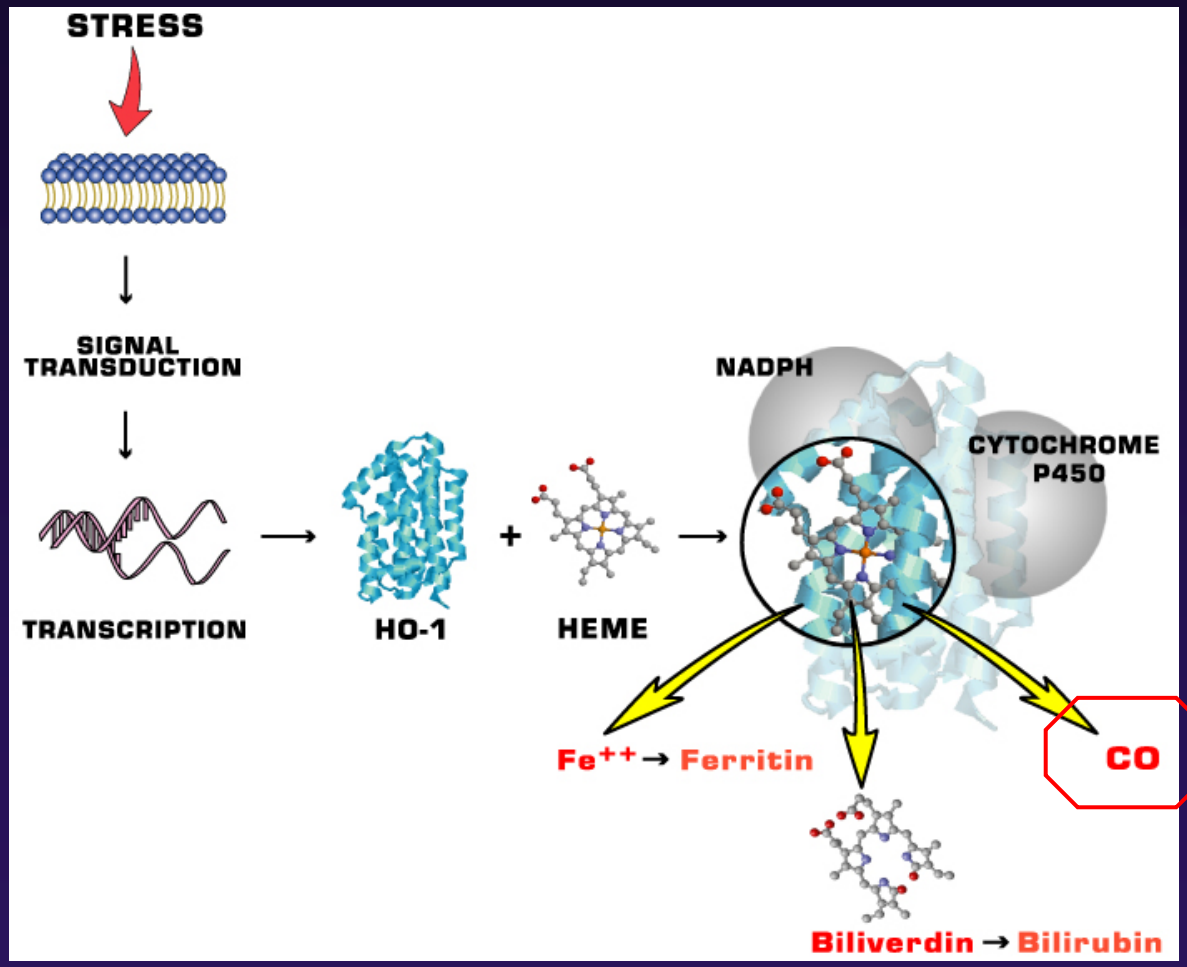
Yachie et al, JCI, 1999. 103:129-35

Human

- Growth retardation.
- Persistent hemolytic anemia.
- Abnormal Coagulation/Fibrinolysis.
- Elevated thrombomodulin and VWF (persistent endothelial damage).
- Endothelium detachment in the glomeruli.
- Iron deposition in renal and hepatic tissue.

HO-1 Deficiency - increased susceptiblity to oxidative stress

Heme Catabolism Yields Bioactive Products: Carbon Monoxide



Hypotheses

Carbon Monoxide (CO) is Responsible for the Effects Observed with HO-1

CO Can Substitute for HO-1 in Eliciting Protection Against Cellular Injury

Model:

Anoxia/reoxygenation-induced apoptosis *in vitro* and ischemia reperfusioninjury in mice.

Role of CO in Vascular Biology

- Morita, T. et.al. Smooth muscle cell-derived carbon monoxide is a regulator of vascular cGMP. *Proc Natl Acad Sci U S A.* 92: 1475, 1995.
- McCuskey, R. S. Does a toxic gas regulate sinusoidal blood flow? *J. Clin. Invest.* 96: 2009, 1995.

CO is an Intercellular Messenger in the CNS

- Verma, A. et.al. Carbon monoxide: a putative neural messenger. *Science* 1993.
- Stevens, C., et. al. Reversal of long-term potentiation by inhibitors of heme oxygenase. *Nature* 1993.
- McKnight, S., et al. NPAS: A Gas-Responsive Transcription Factor. *Science* 2003.

Anti-Inflammatory and Anti-Apoptotic Role of CO

- Sato, K. *et al.* Carbon monoxide generated by heme oxygenase-1 suppresses the rejection of mouse-to-rat cardiac transplants *J Immunol.* 166: 4185, 2001
- Fujita, T., *et al.* Paradoxical rescue from ischemic lung injury by inhaled carbon monoxide driven by derepression of fibrinolysis. *Nat Med.* 7: 598, 2001
- Motterlini, R., *et al.* Carbon monoxide-releasing molecules: characterization of biochemical and vascular activities. *Circ Res.* 90: E17, 2002.
- Moore, B.A., *et al.* Inhaled carbon monoxide suppresses the development of postoperative ileus in the murine small intestine. *Gastroenterology.* 124: 377, 2003.

CO protects against endotoxemia

- Heme oxygenase-1-derived carbon monoxide enhances the host defense response to microbial sepsis in mice. Chung *et al, J Clin Invest*. 118(1):239-47. 2008

- Antimicrobial action of carbon monoxide-releasing compounds. Nobre *et al. Antimicrob Agents Chemother*. 51(12):4303-7. 2007

- Carbon monoxide pretreatment prevents respiratory derangement and ameliorates hyperacute endotoxic shock in pigs. Mazzola *et al, FASEB J*. 19(14):2045-7. 2005

- Carbon monoxide protection against endotoxic shock involves reciprocal effects on iNOS in the lung and liver. Sarady *et al,* FASEB J. 18(7):854-6. 2004

Background: The doses of CO that we encounter daily.

Equivalents

1% (10,000 ppm)	-	Lethal (minutes)
0.3 % (3000 ppm)	-	Pulmonary Function Testing (10 sec)
0.025% (250 ppm)	-	Street Levels
0.01% (100 ppm)	-	Cigarette
0.001% (10 ppm)	-	Ambient
0.035% (35 ppm)	-	US-EPA Guidelines (8 hr workday)

Increase in Carboxyhemoglobin levels are proportional to CO exposure levels in the atmosphere

CO in atmosphere		Estimated COHb in blood (%)
%	ppm	
0.001	10	2
0.007	70	10
0.012	120	20
0.022	220	30
0.035–0.052	350–520	40–50
0.080–0.122	800–1220	60–70
0.195	1950	80

Raub JA, Matieu-Nolf M, Hampson NB, Thom SR. Carbon monoxide poisoning – a public health perspective. . 2000; 145:1-14

Would CO at low concentrations

modulate the inflammatory response?

Is CO effective as a pre-conditioning agent or would the therapy be most beneficial after the onset of injury?

Pre-conditioning

Model: CO Rescues Macrophages From Cell Death Associated With Anoxia/Reoxygenation and Ischemia-Reperfusion Injury.

Hypothesis:

CO increases HIF1α expression *via* a brief burst of ROS in macrophages. HIF1α regulation of TGFβ is then associated with the inhibition of ischemia reperfusion injury in the murine lung.

What is hypoxia inducible factor 1α (HIF1α)?

HIF1α is a transcriptional factor = cellular oxygen sensor.

- Hypoxia - tumors, inflammation, and bacterial killing.
- Cytokines - TNFα, IL-1β, Angiotensin II, Thrombin, PDGF and HER.
- Growth factors - Insulin, IGF-1 & 2, EGF and FGF.

Activated HIF1α translocates into the nucleus

↓

dimerizes with HIF1β

↓

binds to HRE on specific gene promoters

↓

initiates gene transcription-TGFβ

Multifunctional aspect of HIF1α regulated genes.

Angiogenesis: VEGF, FLT-1, TGFβ, PAI-1.

Cell Proliferation: p21, p53, BCL-2, TGFβ1-3, cyclin D1.

Transcriptional regulation: ATF3-4, ETS-1, NUR77.

Cytoskeletal Structure: Vimentin, Keratin 11, 14 & 19.

Motility: TGFα, Glucose-6-Isomerase(GPI).

Vasomotor Tone: ET-1. iNOS, HO-1, Bach-1, ANP

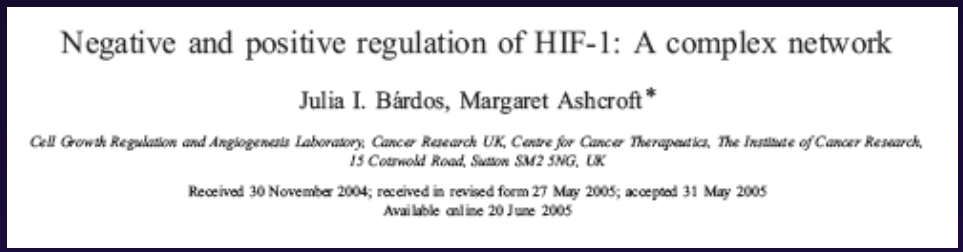
ECM: Collagen V, Fibronectin, Cathepsin D, MMP2.

Metabolism: GAPDH, GLUT1 & GLUT3, PFKL, PFKB3, PGK1.

Inflammation: COX-2, CD18, CYP4B1.

Erythropoeisis: EPO, Transferrin & Transferrin receptor

Cellular regulators of HIF1α



Negative and positive regulation of HIF-1: A complex network

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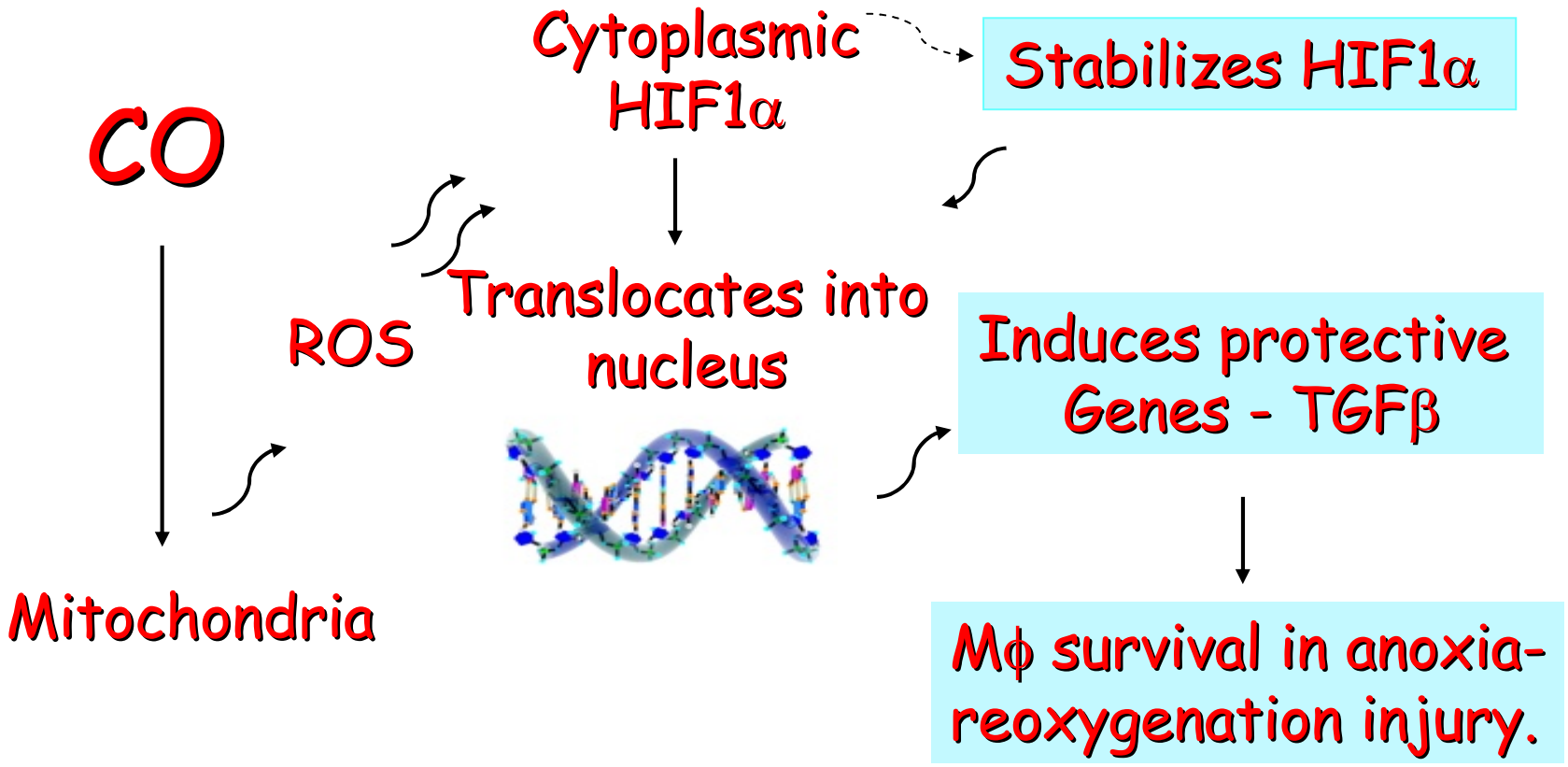
Received 30 November 2004; received in revised form 27 May 2005; accepted 31 May 2005
Available online 20 June 2005

Negative	*Positive*
• PHDs	• Hypoxia
• VHL	• p300
• FIH	• P13K/Akt
• pTEN	• MAPK
• p53	• NO
• GSK3β	• ROS

Does administration of exogenous low dose
CO induce the generation of "tightly regulated ROS production"
in order to activate HIF1α?

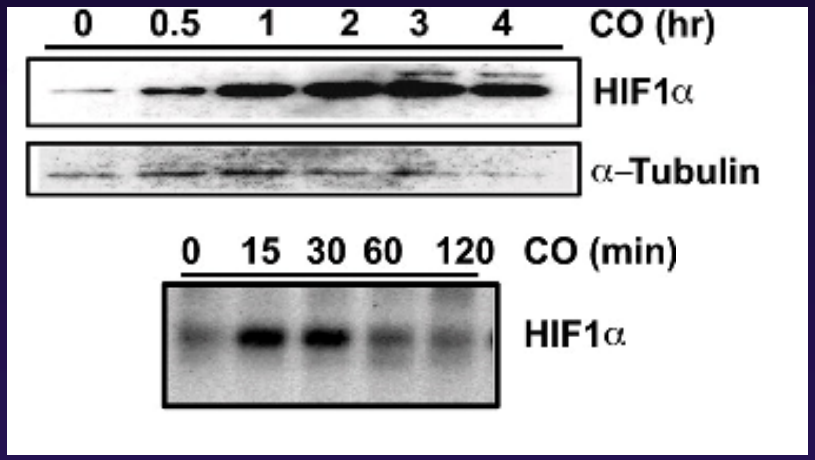
Uncontrolled ROS = negative effect on cells
Tightly regulated ROS = serve as "signaling molecules"

Model of HIf1α stabilization by low dose CO in macrophages



Induction of HIF-1α stabilization: expression and activity in macrophages after administration of CO.

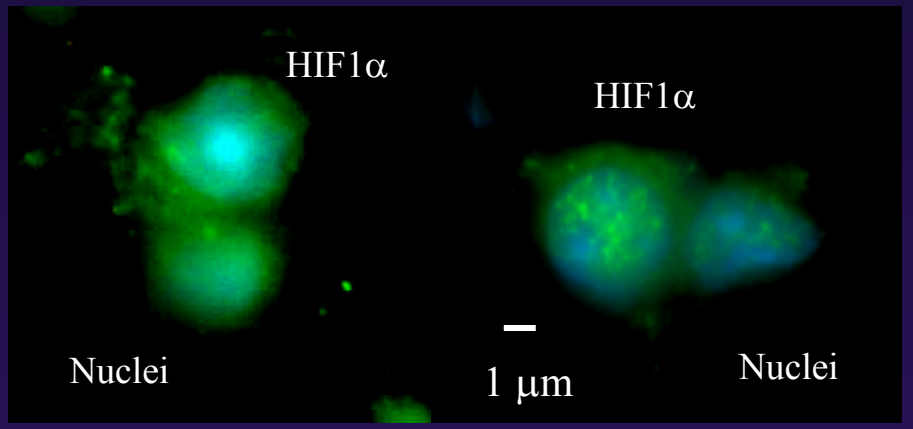
Western blot



EMSA

CO treated in situ lung macrophages

Air



3D-reconstructed isolated lung macrophages

(Scale bar: 1 μm.)

How does CO stabilize HIF1α? Targeting mitochondria hemoproteins to generate a secondary messenger-ROS.

CO binds to Fe++ containing hemoproteins

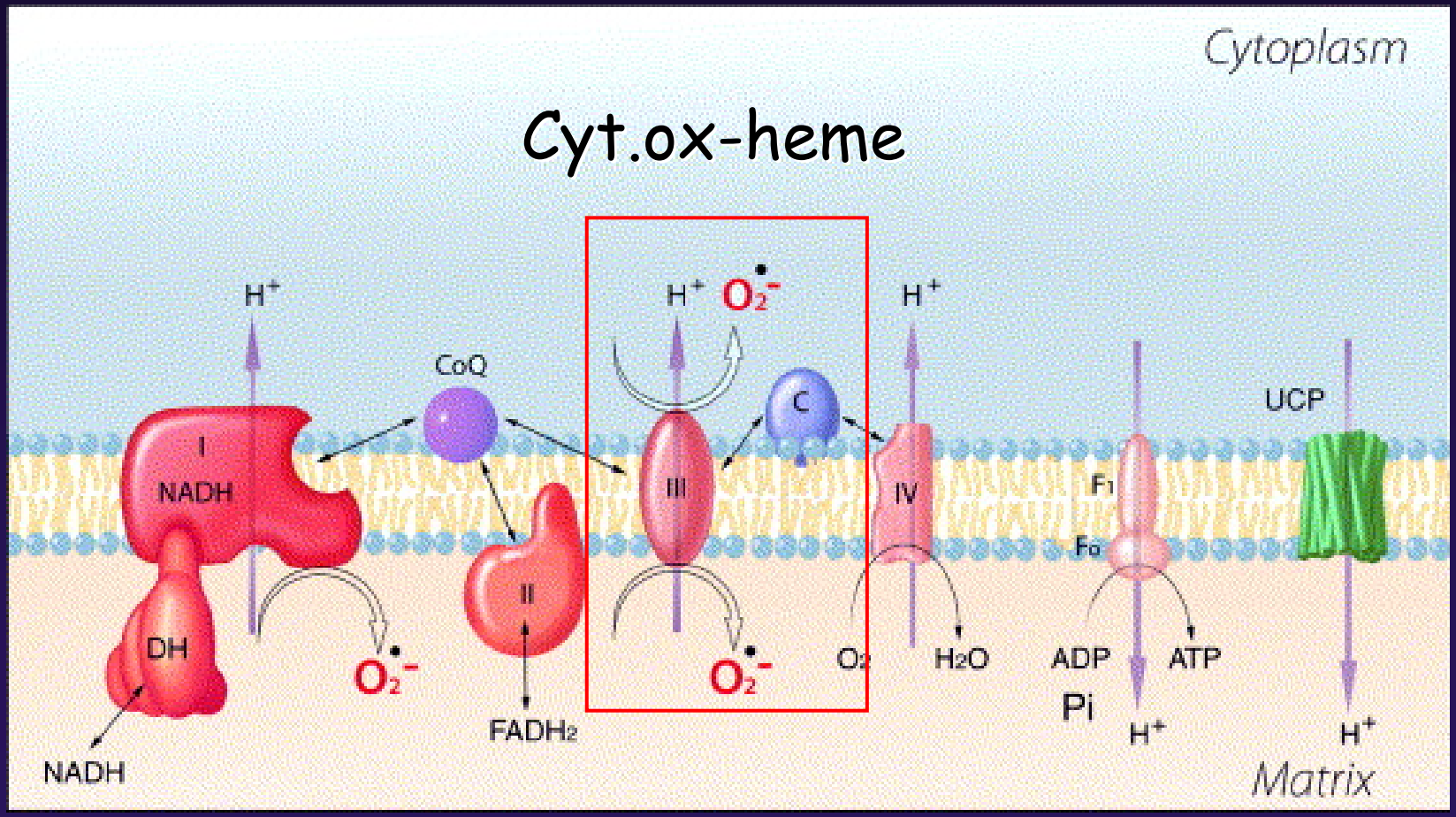
Fe2+ Heme group

Protein

Examples

Guanylate Cyclase	NADPH Oxidase
Nitric Oxide Synthases	Cytochrome p450
Cyclooxygenase	NPAS2/BACH-1
Catalase	Cytochrome Oxidase - Mitochondria Transport Chain

Binding of mitochondria by CO induces ROS: complex III



Balaban, et al., *Cell* 2005, 120:483-495

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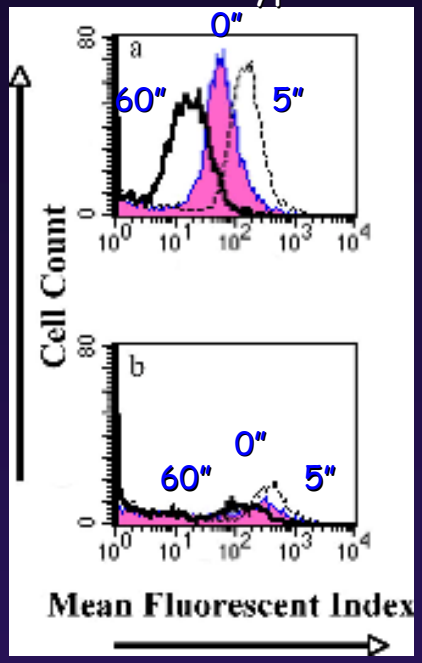
Exposure to CO induces ROS burst in macrophages

Wild-type



Mitochondria deficient cells (ρ^0)

Green
← ROS release

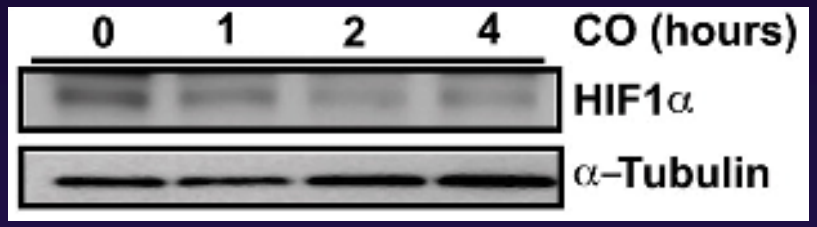
Red
← Mitotracker

QuickTime™ and a decompressor are needed to see this picture.

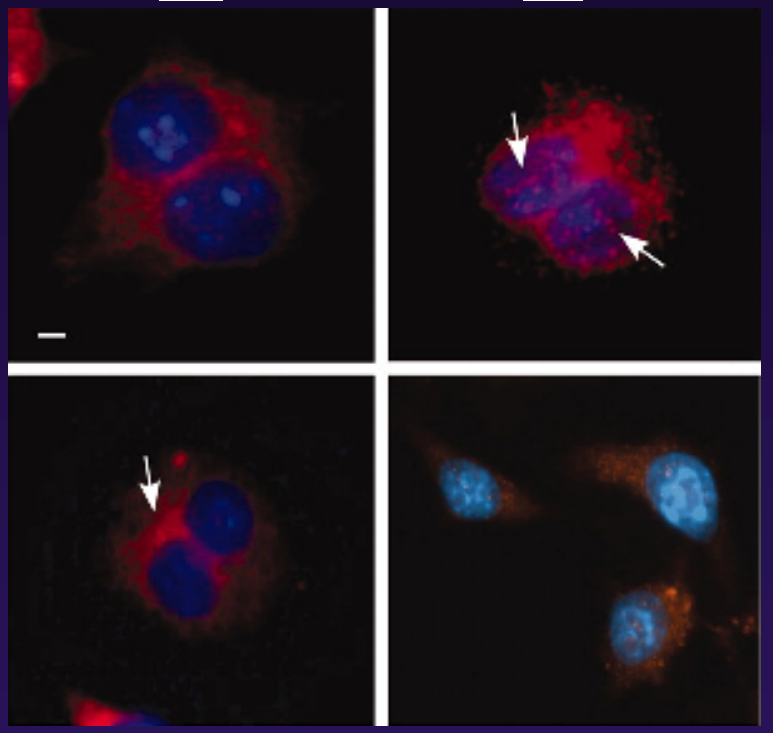
Mitotracker → *Load cells* / *DCF dye* → *15 min* → *Treat cells with* / *CO releasing molecules* → *min*

Mitochondria-Derived ROS Mediate CO Induced HIF-1α Activity

ρ⁰ cells exposed to CO



Immunofluorescent staining for HIF-1α



HIF-1α = red, denoted by arrows
Nuclei = blue

Does CO induction of HIF1α result in a physiological function? Activity of TGFβ.

Proc. Natl. Acad. Sci. USA 2007 104, 5109-5114

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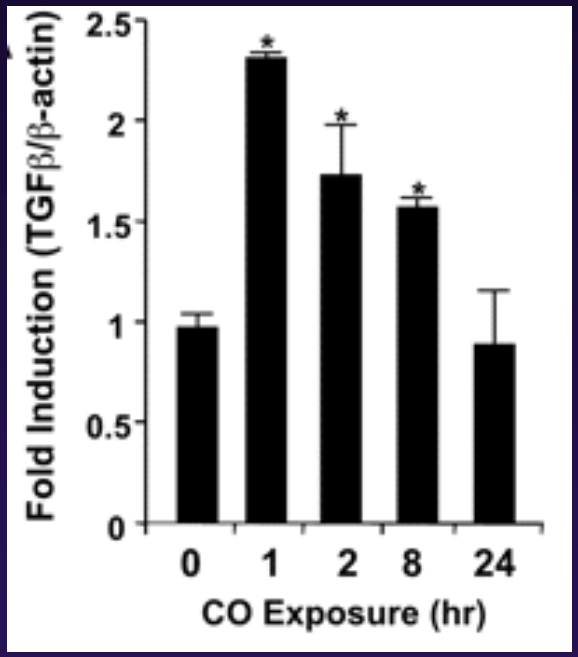
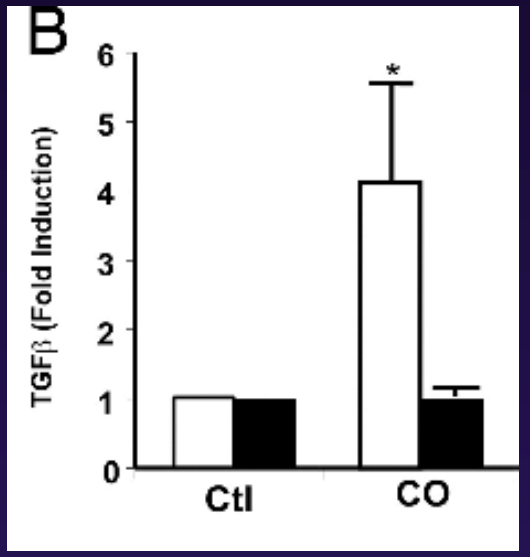
CO Induces the Expression of TGF-β, dependence on HIF1α

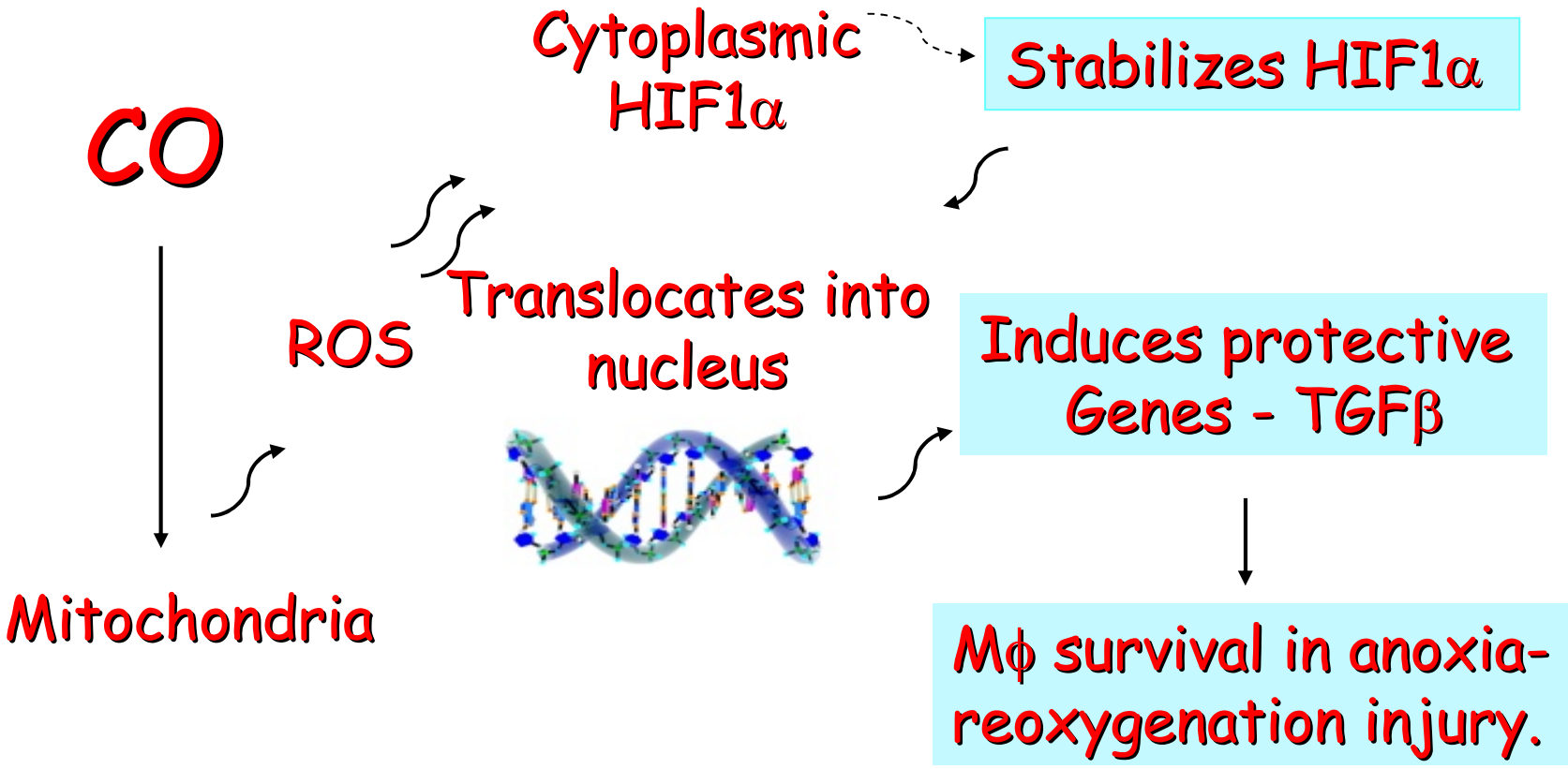


Kinetics of TGF-β mRNA expression



LMP vector control cells = open bars
HIF-1α-miRNA cells = filled bars

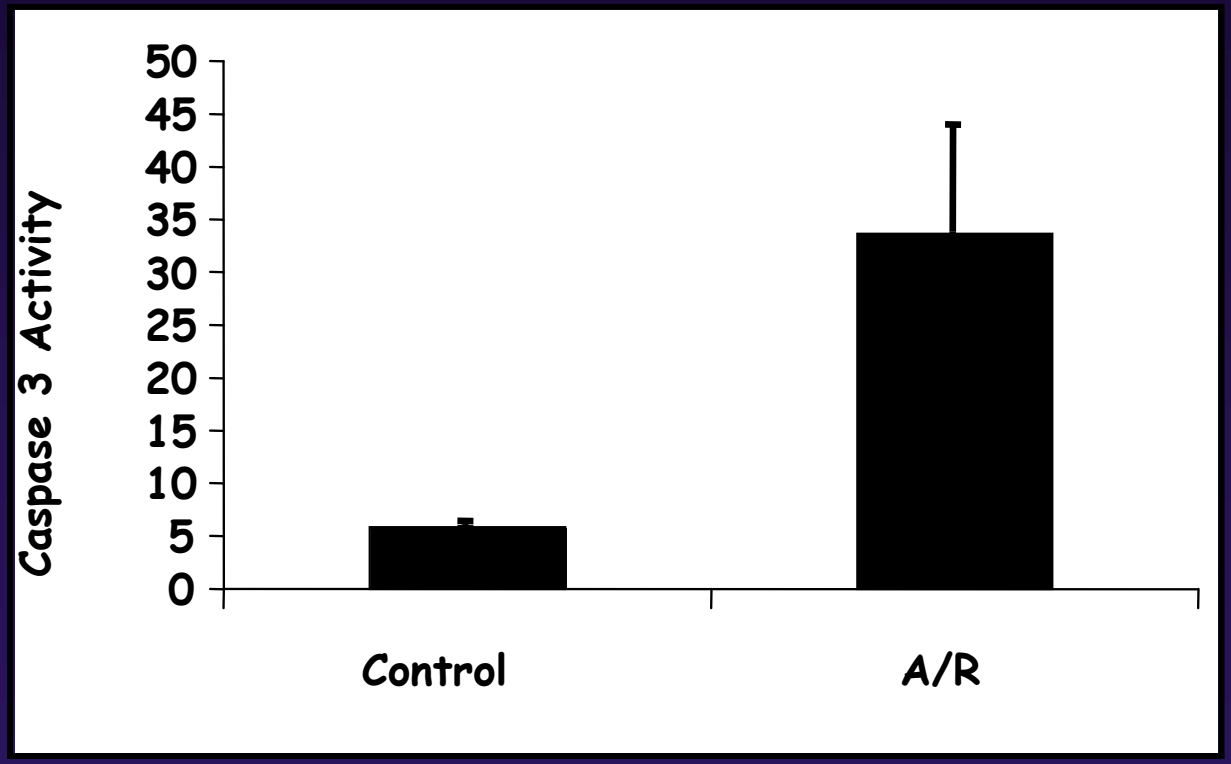
Model of HIf1α stabilization by low dose CO in macrophages



Establishing a model of Anoxia/Reoxygenation to demonstrate HIF1α function in IRI

Anoxia → *8 hr* → *Re-oxygenation* → *16 hr*

0.1% O_2 *21% O_2*



Anoxia/Reoxygenation induces apoptosis in macrophages

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Proc. Natl. Acad. Sci. USA 2007 104, 5109-5114

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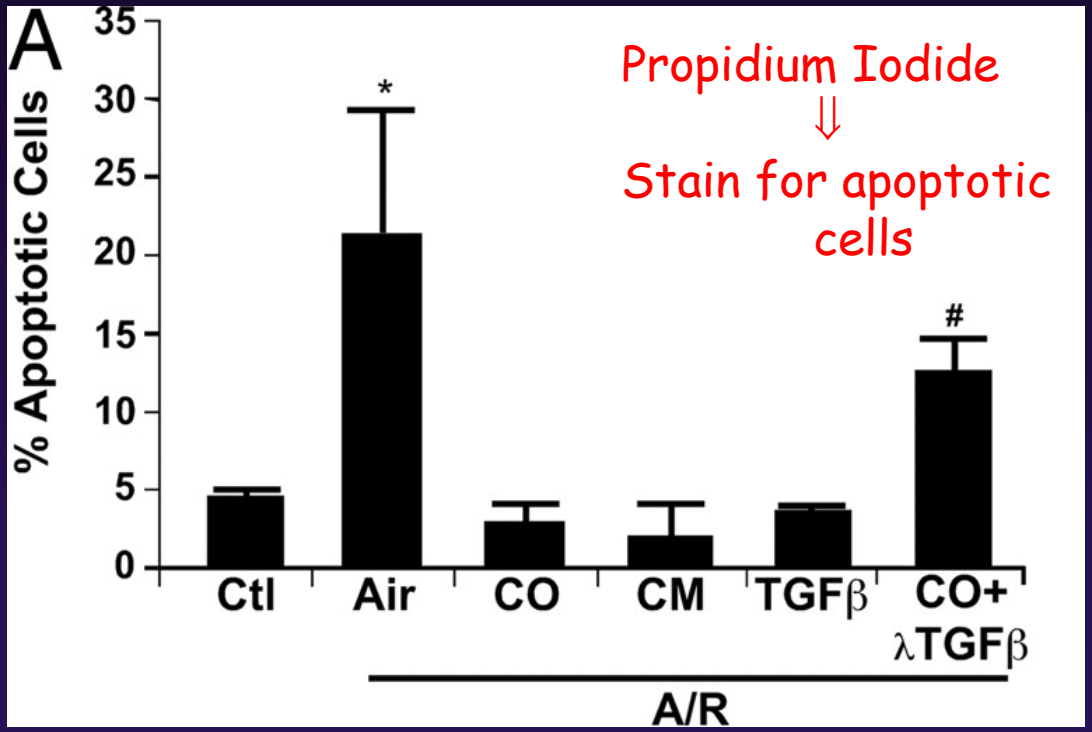
CO and Conditioned Media from CO-exposed mϕ prevents A/R-induced apoptosis



CM = condition media from mϕ exposed to 24 hr CO

Soluble factor that is secreted from macrophages exposed to CO is responsible for cytoprotection against A/R -TGFβ

Proc. Natl. Acad. Sci. USA 2007 104, 5109-5114

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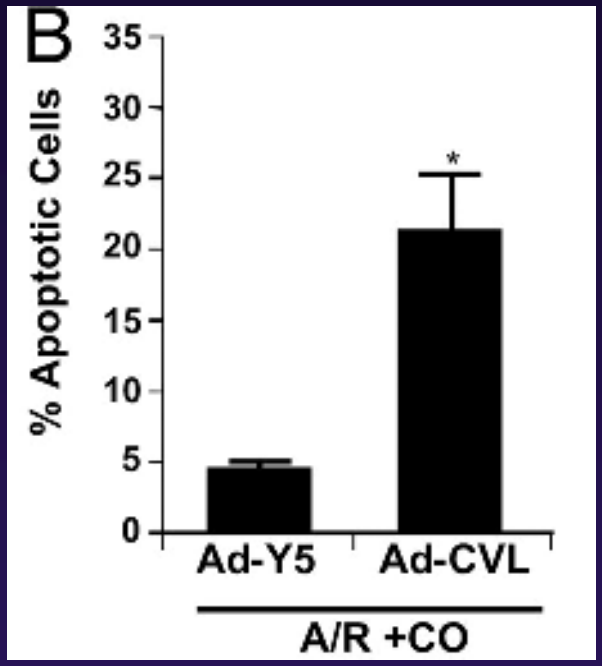
Bone marrow derived macrophages from HIFLoxP mice x AdCre (HIF1α $^{-/-}$) apoptose after Anoxia/Reperfusion treatment



Ad-CVL = BMDM from HIF-1α-Loxp mice + Ad-Cre)
Ad-Y5 = vector control

Macrophage survival is contingent upon both HIF1α and TGFβ

Proc. Natl. Acad. Sci. USA 2007 104, 5109-5114

Model

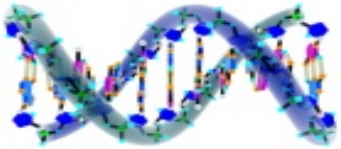
CO

Cytoplasmic HIF1α

Stabilizes HIF1α

ROS

Translocates into nucleus



Induces protective Genes - TGFβ

Mitochondria

Mϕ survival in anoxia-reoxygenation injury.

Does the in vitro macrophage model of anoxia/reoxygenation translate to an in vivo situation in the lung during ischemia-reperfusion injury?

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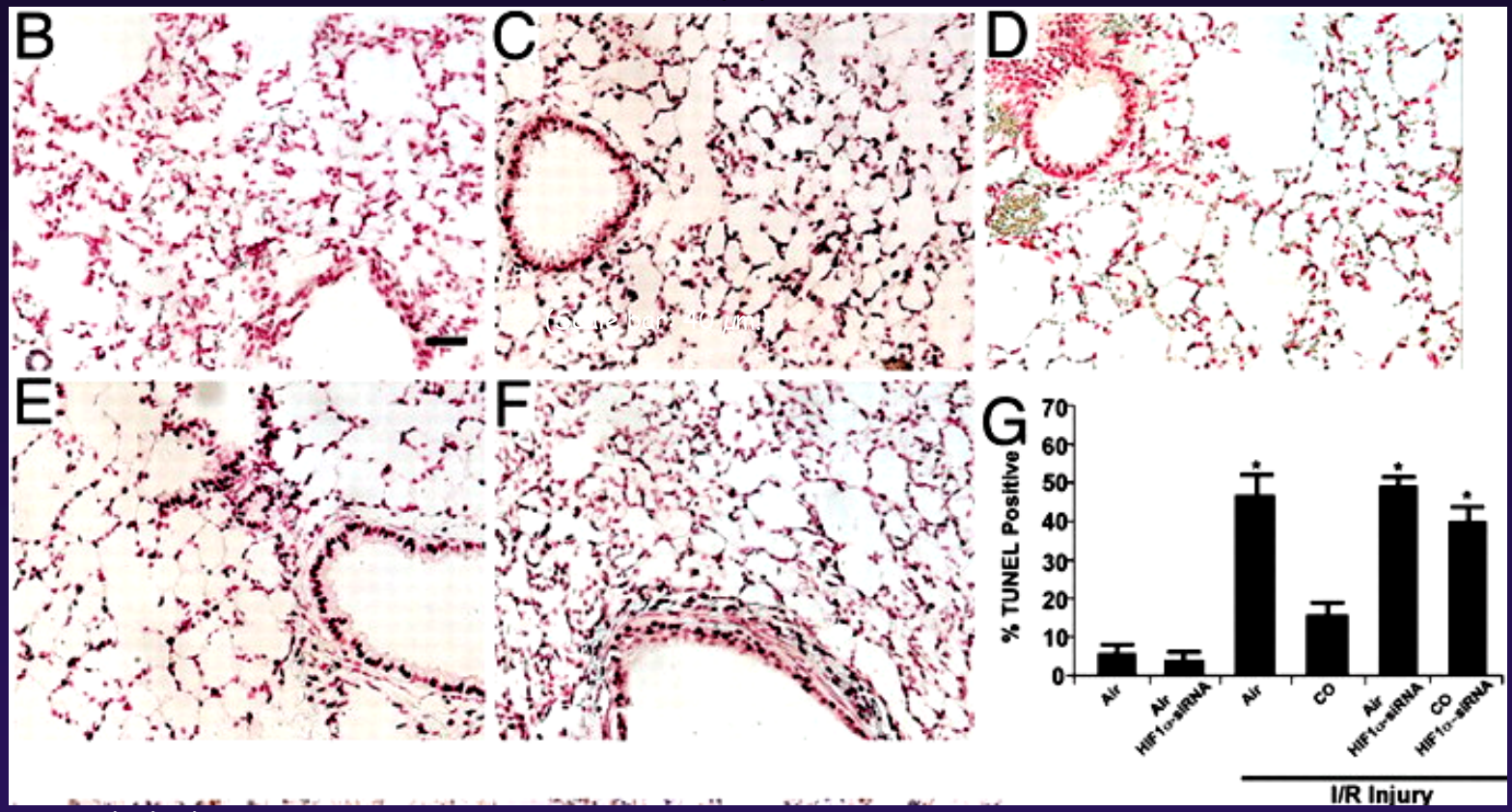
Silencing of HIF-1α abrogates CO-induced protection against lung IRI.

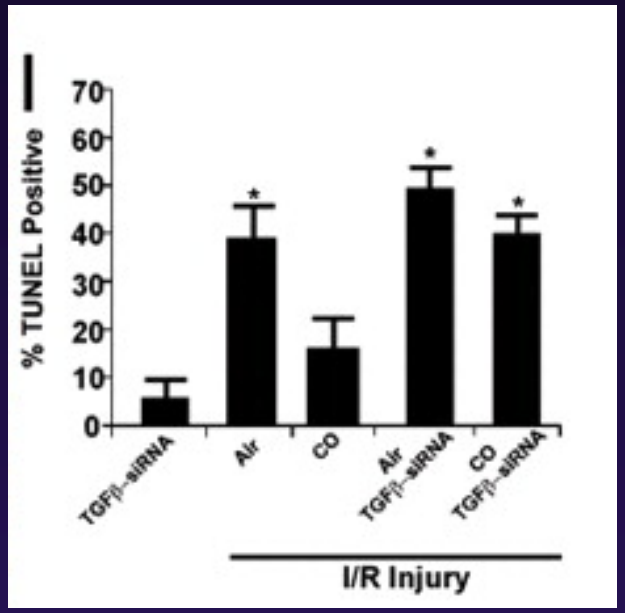


HIF-1α-siRNA → CO or Air (1hr) → IRI (48hr) → TUNEL analyses

or saline (24-48h)

(Scale bar: 40 μm.)

TGF-β expression by CO is required for protection



CO was unable to protect against IRI in mice treated with TGF-β-siRNA

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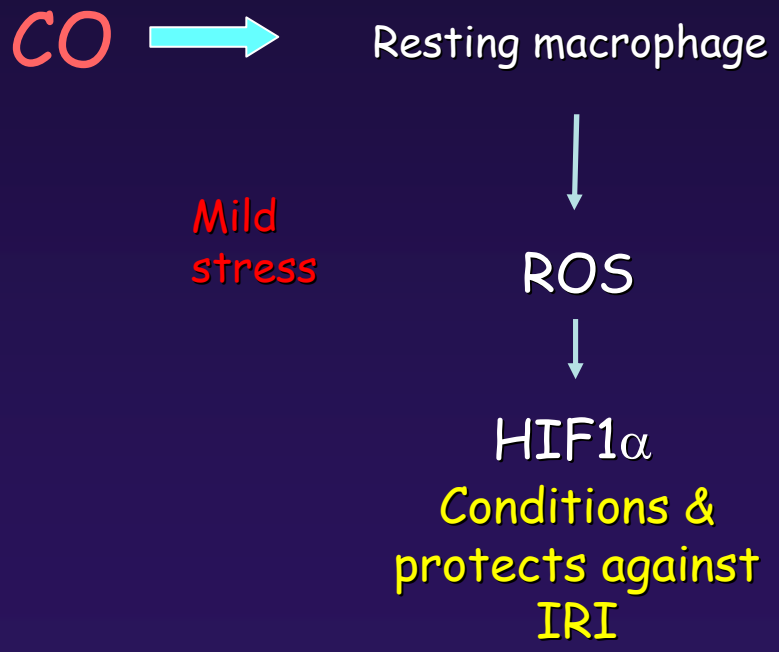
Summary

- CO induces a transient burst of ROS that triggers the expression of TGFβ in macrophages.
- Expression of TGFβ by CO requires mitochondria-derived ROS and HIF1α.
- CO-conditioned media rescues macrophages from A/R-induced cell death acting *via* TGFβ expression.
- In a lung IRI model, administration of HIF1αsiRNA decreased macrophage expression of TGFβ which resulted in increased cell death and loss of CO protection.
- TGFβ is a mediator of CO-induced cytoprotection in IRI.

Innate Response of the Macrophage (Pre-treatment)

1. CO acts rapidly on heme containing proteins (mitochondria)
2. ROS activate selective signaling molecules
3. In the presence of a stimulus, CO acts to amplify a response (pos or neg)
4. CO reestablishes homeostasis accordingly



Reported Effects of CO

Beneficial

- Transplantation
- Acute Lung Injury
- Pulmonary Fibrosis
- Pulmonary Hypertension
- Paralytic Ileus
- Ischemia/Reperfusion Injury
- Colitis
- Shock (endotoxin/hemorrhagic)
- Tumor Growth/Angiogenesis
- Autoimmune Disorders

Ineffective

- Rhabdomyolysis
- Pancreatitis
- Dextran Sulfate-Induced IBD

Questions for the future: CO as a therapeutic?

- Specificity of cell and tissue type; VSM, EC and macrophages.

- Dose effect--protection versus toxicity; 10-500 ppm *vs* higher doses.

- Mechanism(s) of action/Cellular Targets; ROS, hemoproteins, HIF1α and TGFβ.

Acknowledgement

BIDMC/
Harvard Medical School

Leo Otterbein
Tess MacDonald
Dave Gallo
Hideyashu Sakihama
Michael Thomas
Hong Jun Wang
Soo Sun Lee

Basia Wegiel
Silvia Mazzola
Martin Bilban
Fritz Bach

Yale University

Patty Lee
Ge Jiang

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