HYPOXIA

Cellular adaptation to physiological and pathological stresses

Sylvain Provot, Ph.D.
CR1 INSERM, "Atip-Avenir" team leader
at Hôpital Lariboisière, Paris.
sylvain.provot@inserm.fr

Hypoxia - Definitions

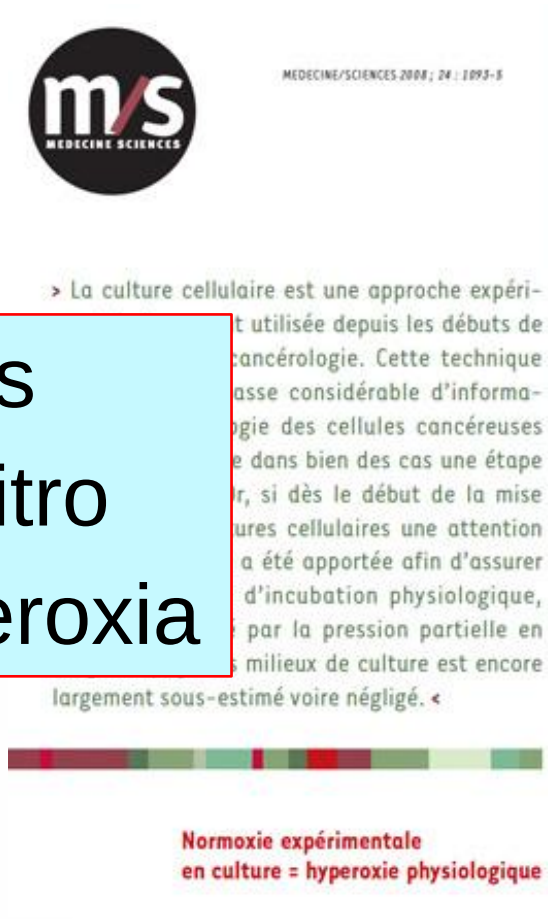
- Lack/deprivation of oxygen
- “Hypoxia” opposes “Normoxia” (normal levels of O_2)
- What is “Normoxia”?

Atmospheric O_2 level 21%	*vs*	O_2 levels in tissues 3-5%

Normoxia in organisms ≠ 21% O_2!

Hypoxia - Definitions

For all cells
cultured in vitro
21% O_2 = hyperoxia



m/s MEDECINE SCIENCES

MEDECINE/SCIENCES 2008 ; 24 : 1093-5

Pression partielle en oxygène et culture de cellules cancéreuses

Un demi-siècle d'artéfacts ?

Didier Wion, Maurice Dematteis, Marie-France Nissou, Céline Cretallaz, François Berger, Jean-Paul Issartel

› La culture cellulaire est une approche expéri-
t utilisée depuis les débuts de
cancérologie. Cette technique
asse considérable d'informa-
gie des cellules cancéreuses
e dans bien des cas une étape
r, si dès le début de la mise
ures cellulaires une attention
a été apportée afin d'assurer
d'incubation physiologique,
par la pression partielle en
milieux de culture est encore
largement sous-estimé voire négligé. ‹

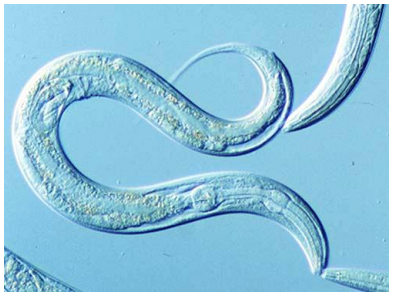
Normoxie expérimentale en culture = hyperoxie physiologique

D. Wion, M.F. Nissou, J.P. Issartel : Inserm U836, Équipe 7, Institut des Neurosciences, Bâtiment Edmond J. Safra des Neurosciences, chemin Fortuné Ferrini, Site Santé, 38706 La Tronche Cedex, France.
didier.wion@ujf-grenoble.fr

Hypoxia - Introduction

- O_2 started accumulating on Earth ~2.5 billion years ago
- "Adaptive evolution to oxygen" precedes "adaptive mechanisms to hypoxia"

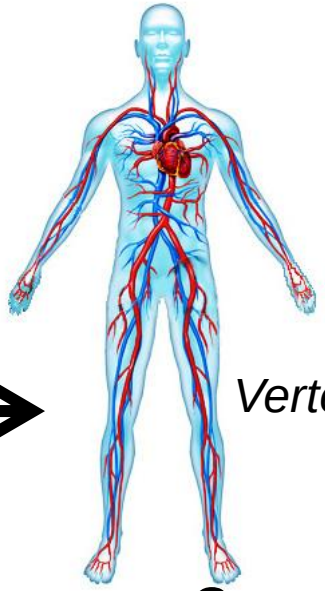
C. Elegans



O_2 provided by diffusion

Drosophila

Tracheal system (branching tubules) brings O_2 into the body



Vertebrates

Lungs + Complex cardiovascular system (Red blood cells)

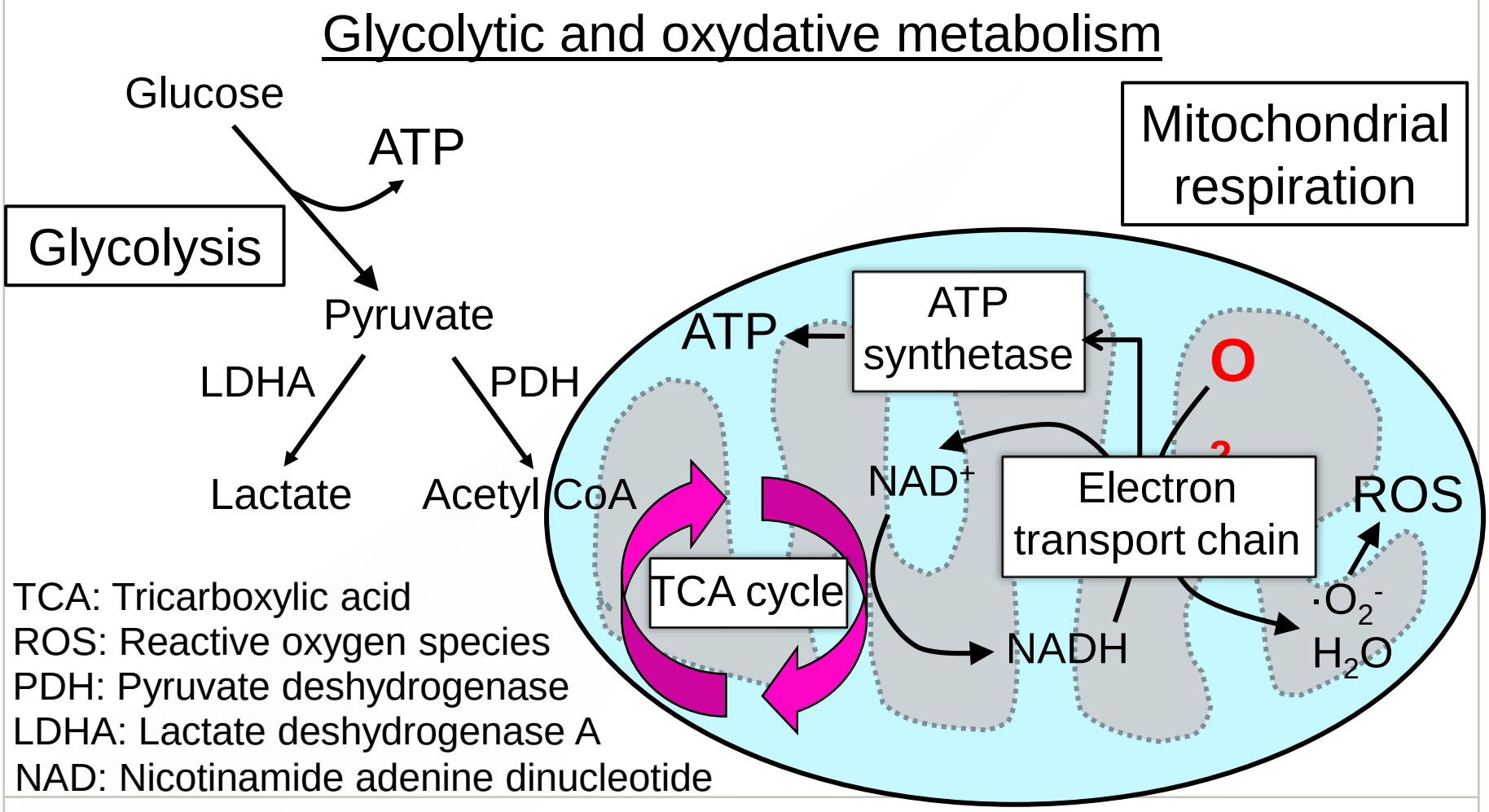
Evolution

Hypoxia - Introduction

- Increased atmospheric O_2 ➔ oxidative phosphorylation
- Oxidative phosphorylation ➔ high-energy ATP production

Energy produced by oxydative metabolims (mitochondrial respiration) > Energy produced by glycolytic metabolims (anaerobic)

Hypoxia - Introduction



Hypoxia - Introduction

- What is the downside of using oxydative phosphorylation?
  - ⇨ Production of ROS ⇨ Cell death
- What are the risks associated with hypoxia?
  - ⇨ Increased production of ROS ⇨ Cell death
  - ⇨ Lack of energy (↓ ATP)

Cellular adaptation to hypoxia

1- Produce energy:

⇨ Increased anaerobic glycolysis ⇨ ATP (not a lot)

2- Maintain cell survival:

⇨ Counteract ROS production (mitochondrial autophagy)

3- Increase O_2 delivery :

⇨ Erythropoiesis (↑EPO) ⇨ More red blood cells

⇨ Angiogenesis (↑VEGF) ⇨ More blood vessel

O_2 = a regulatory signal

The O_2-sensing machinery

The Hypoxia Inducible Factors

- HIFs = bHLH transcription factors
- HIFs = heterodimers of HIFα and HIFβ subunits
- The “alpha” subunit is O_2-labile and very unstable
- The “beta” subunit is very stable and present in excess
  ⇨ Hifα protein levels determine HIF transcription activity
- There are 3 isoforms of HIFα in mammals

The O_2-sensing machinery

The Hypoxia Inducible Factors

- Hif-1α is ubiquitously expressed
- Hif-2α expression is restricted to certain tissues
  (Lungs, kidneys, liver, vascular endothelial cells, etc.)
- Hif-3α expression is also restricted
  Hif-3α lacks a transactivation domain
  Some splice variants inhibit Hif-1α and Hif-2α activities

The O_2-sensing machinery

The Hypoxia Inducible Factors

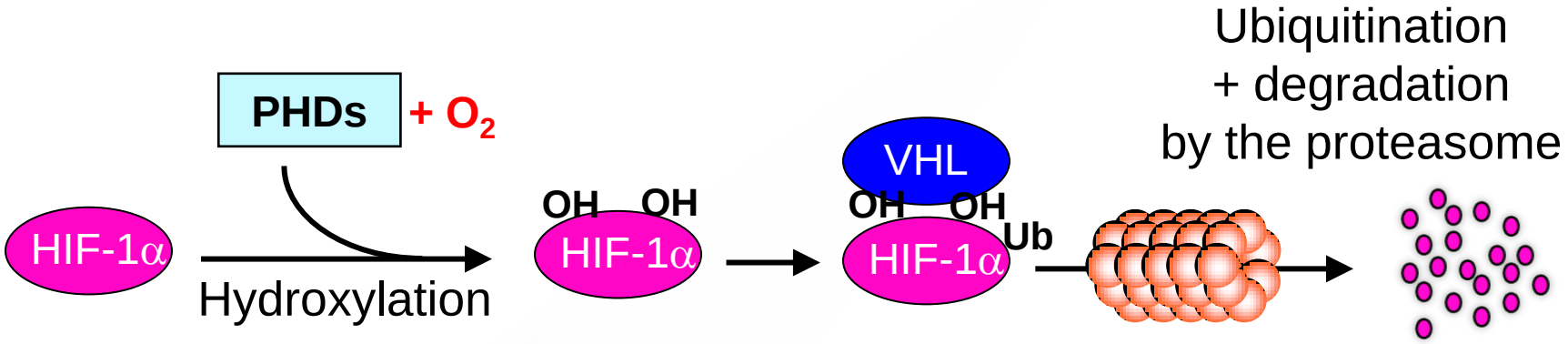
- Hif-1α regulates most of the genes induced by hypoxia

⇨ Hif-1α = Key factor for cellular adaptation to hypoxia

- Hif-1α half-life = ~ 5 min
- Hif-1α protein stabilization begins at O_2 concentrations < 6%
- Exponential increase of Hif-1α protein levels with increasing hypoxia levels

The O_2-sensing machinery

Hif-1α is degraded in presence of oxygen

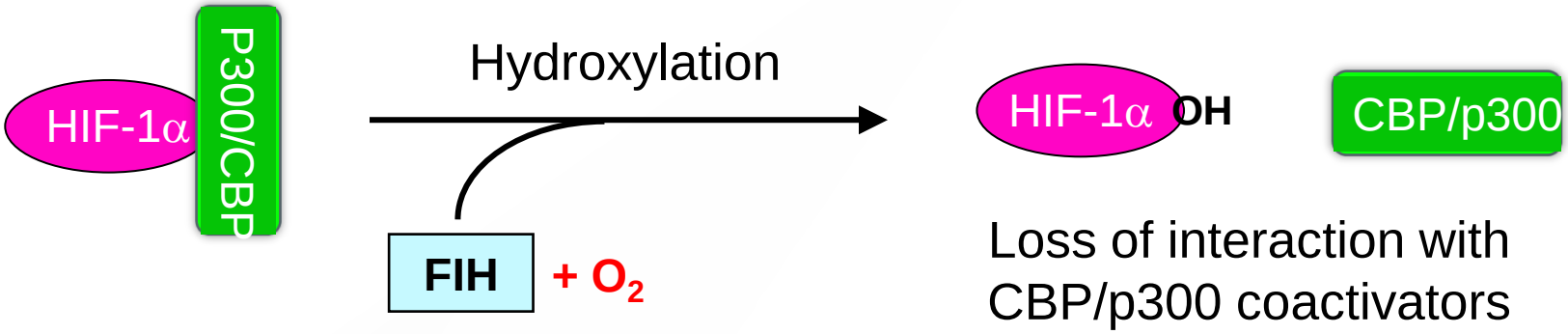


PHD: Proly Hydroxylase Domain protein

VHL: von Hippel Lindau tumor suppressor = E3 ubiquitin ligase

The O_2-sensing machinery

Hif-1α mediated transcription is inhibited in presence of oxygen



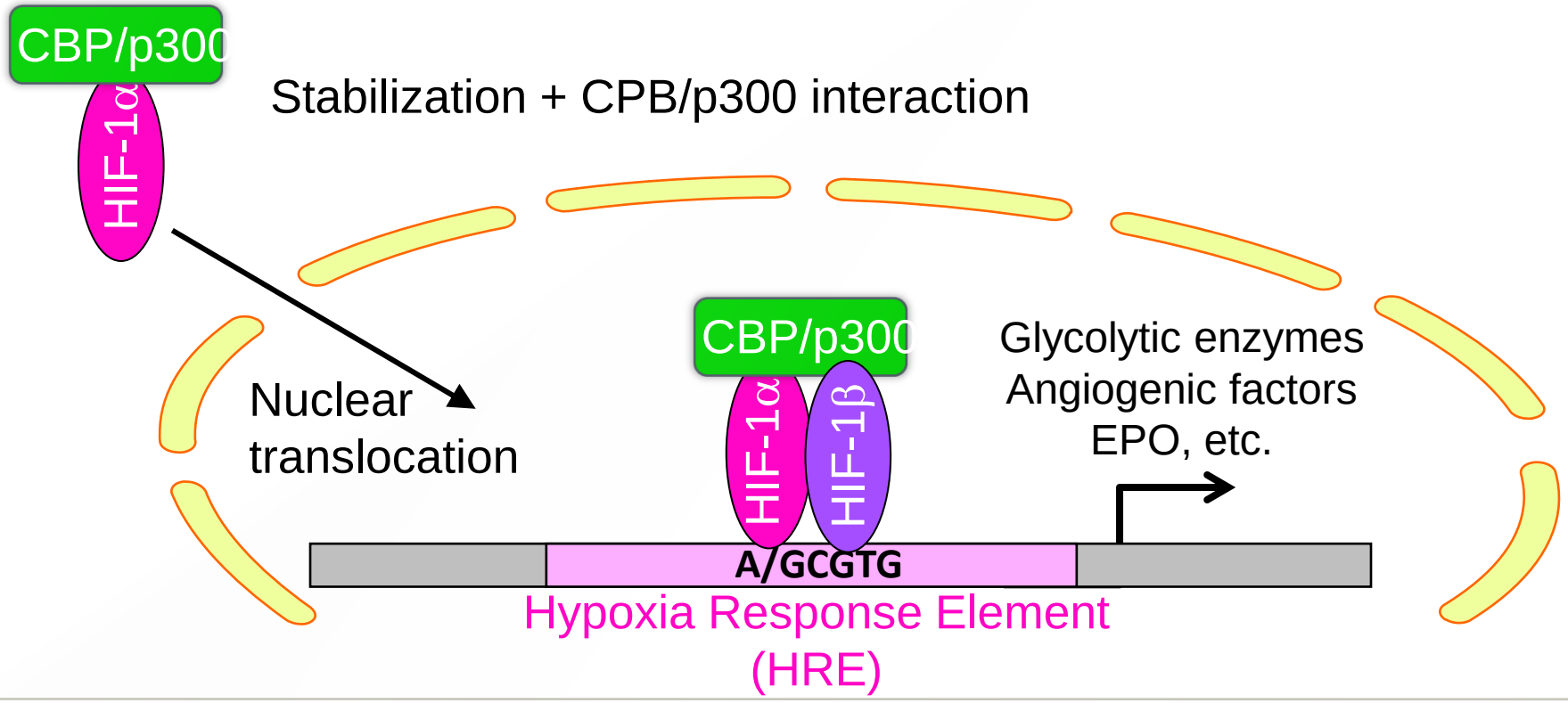
FIH: Factor Inhibiting HIF = asparaginyl hydroxylase

p300 = E1A binding protein p300

CBP = CREB-binding protein

The O_2-sensing machinery

Hif-1α is stabilized in hypoxic conditions

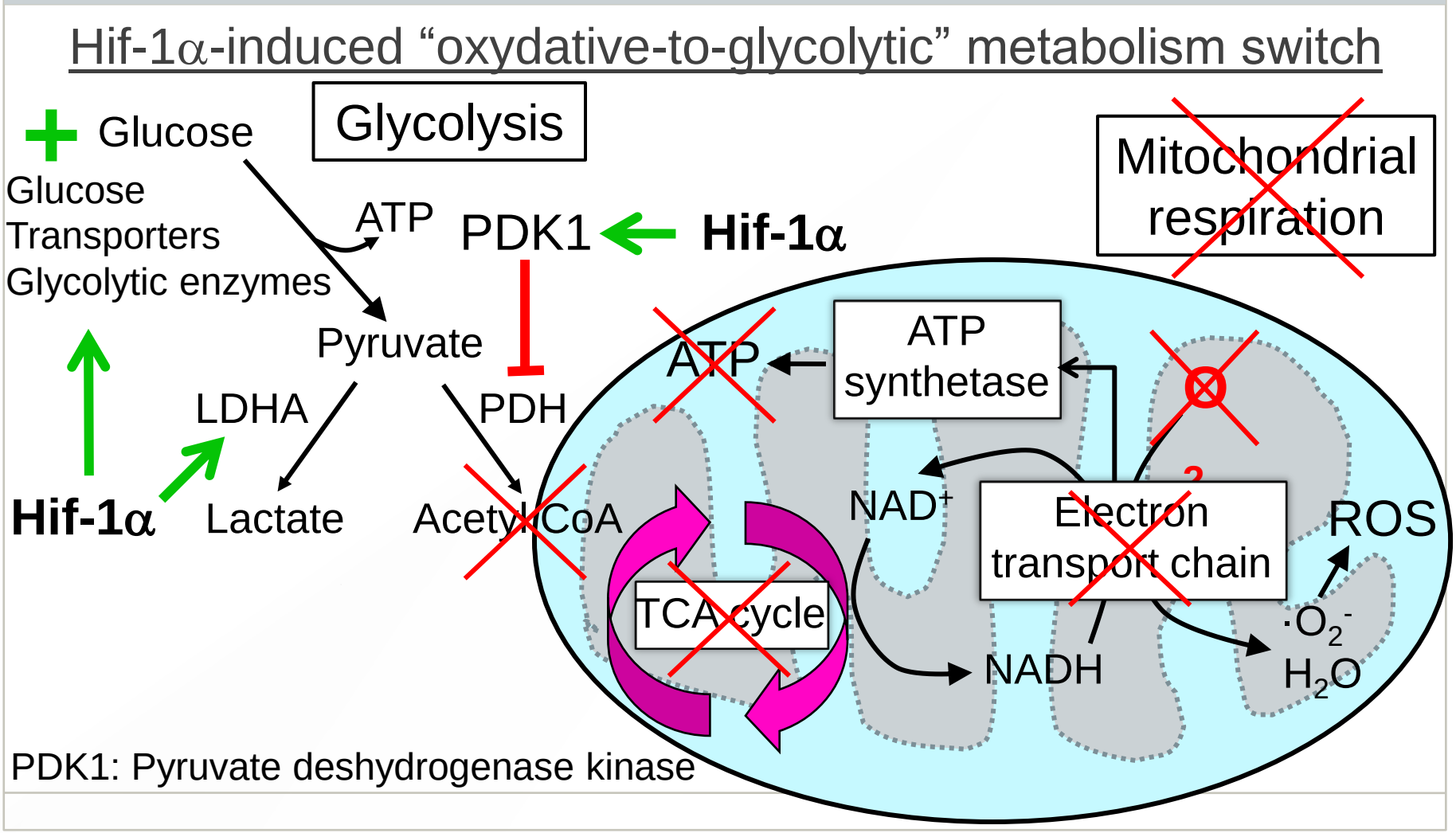


Cellular adaptation to hypoxia

1- Produce energy:

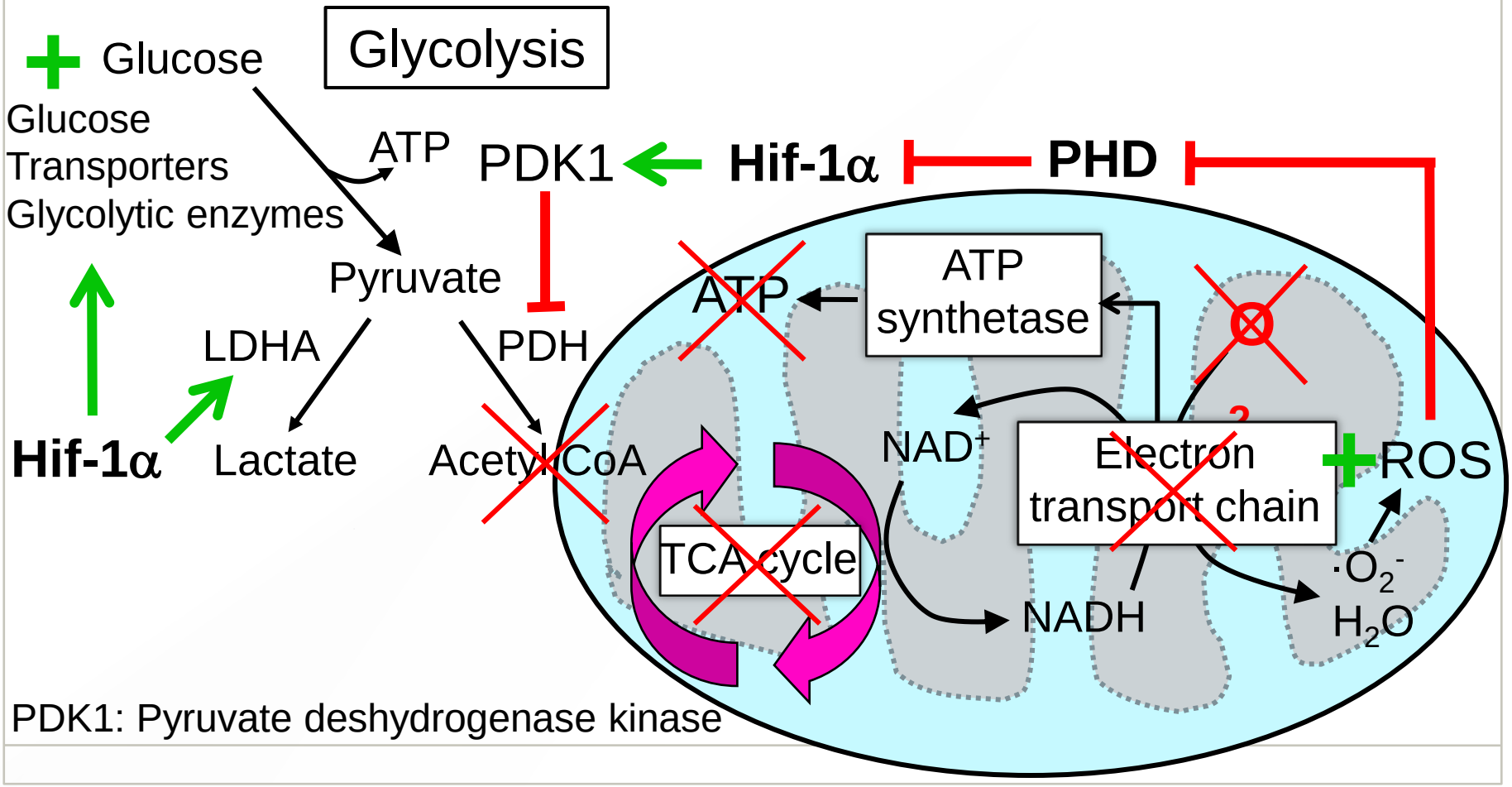
⇨ Increased anaerobic glycolysis ⇨ ATP (not a lot)

Cellular adaptation to hypoxia



Cellular adaptation to hypoxia

Hif-1α-induced “oxydative-to-glycolytic” metabolism switch



PDK1: Pyruvate deshydrogenase kinase

Cellular adaptation to hypoxia

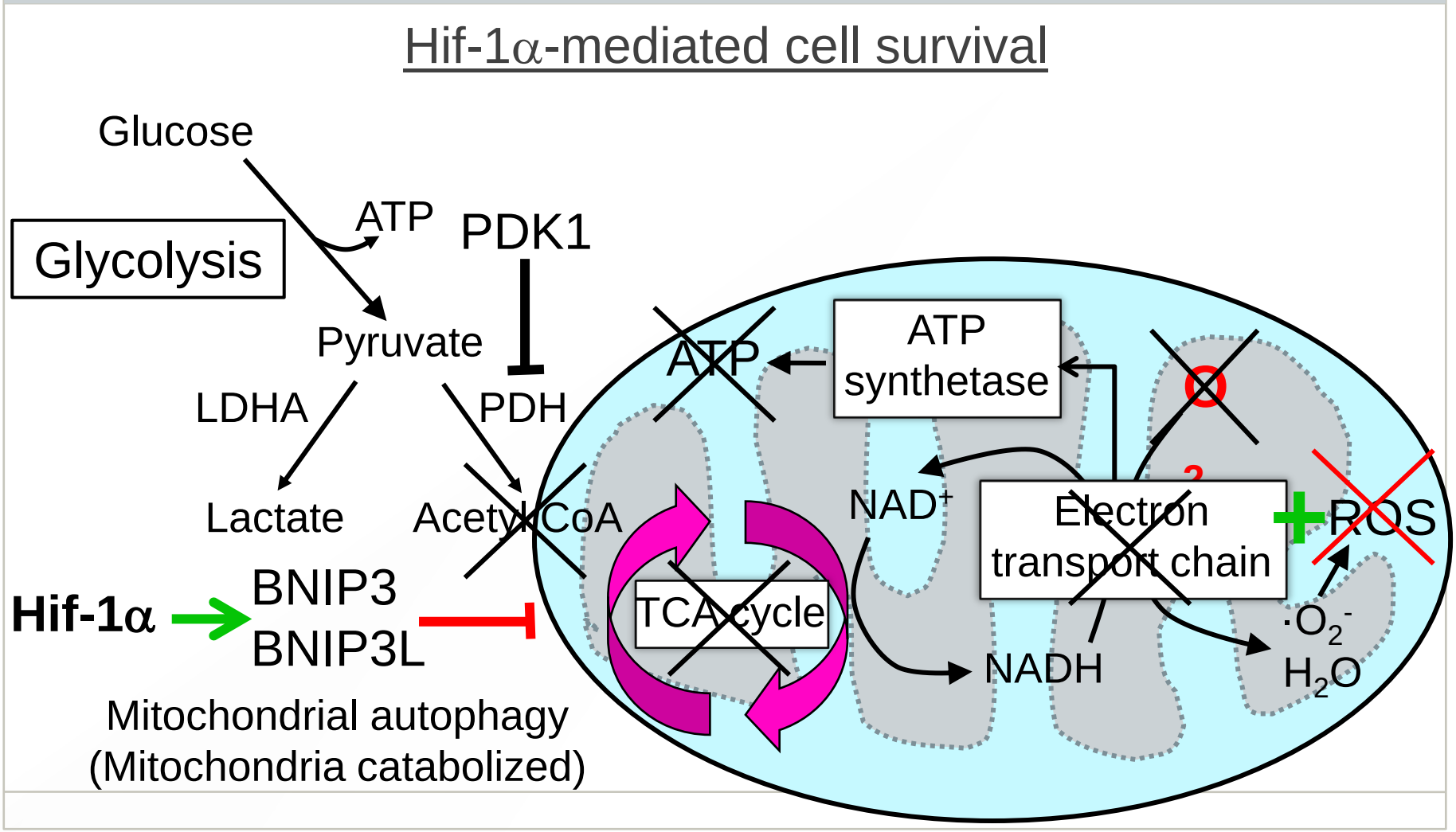
1- Produce energy:

⇨ Increased anaerobic glycolysis ⇨ ATP (not a lot)

2- Maintain cell survival:

⇨ Counteract ROS production

Cellular adaptation to hypoxia



Cellular adaptation to hypoxia

1- Produce energy:

⇨ Increased anaerobic glycolysis ⇨ ATP (not a lot)

2- Maintain cell survival:

⇨ Counteract ROS production

3- Increase O_2 delivery :

⇨ Erythropoiesis (↑EPO) ⇨ More red blood cells

⇨ Angiogenesis (↑VEGF) ⇨ More blood vessel

O_2 = a regulatory signal

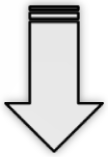
Physiological roles of hypoxia

O_2 concentration in adult tissues = 3-5%

and in embryos: 1-5%

AND

Hif-α is stabilized at O_2 levels < 6%

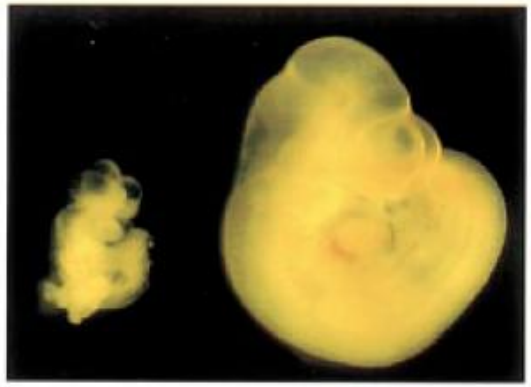


HIFs play physiological roles in most (all?) tissues

Physiological hypoxia

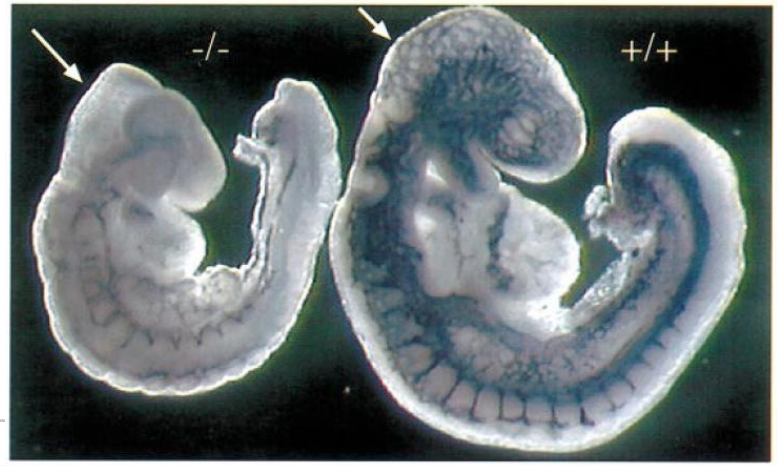
Hif-1α knockout mice

- Die at ~ED 10.5
- Cardiac malformations
- Vascular defects
- Impaired erythropoiesis
- Missing somites



E9.5

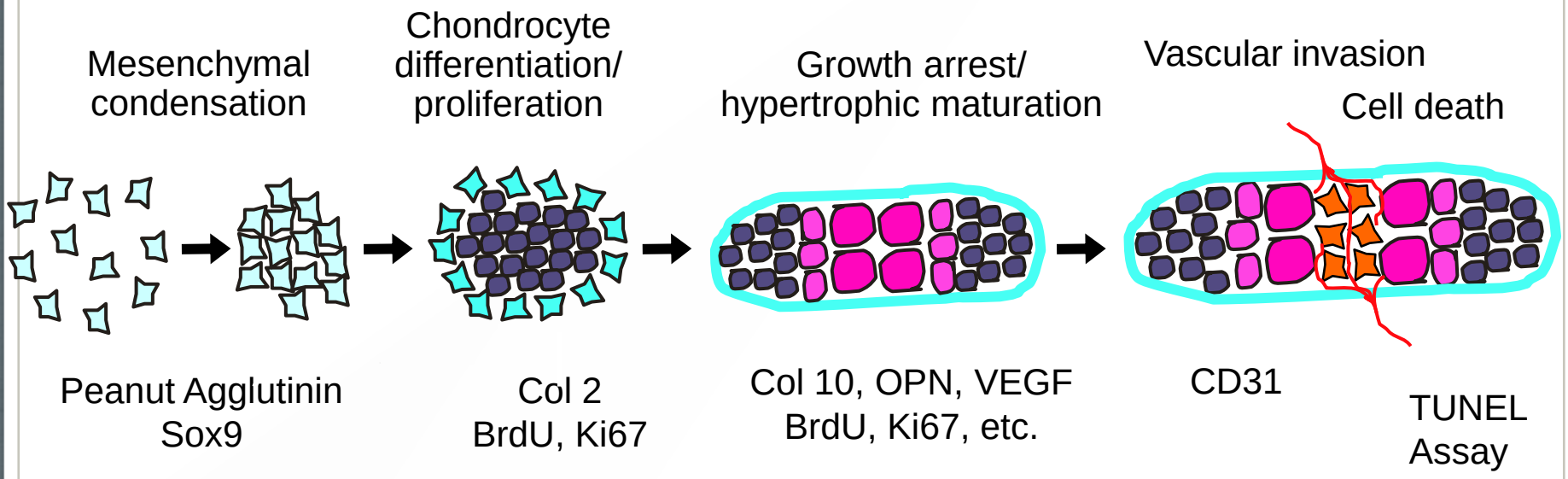
-/- +/+



Physiological hypoxia

Role of Hif-1α in skeletal development

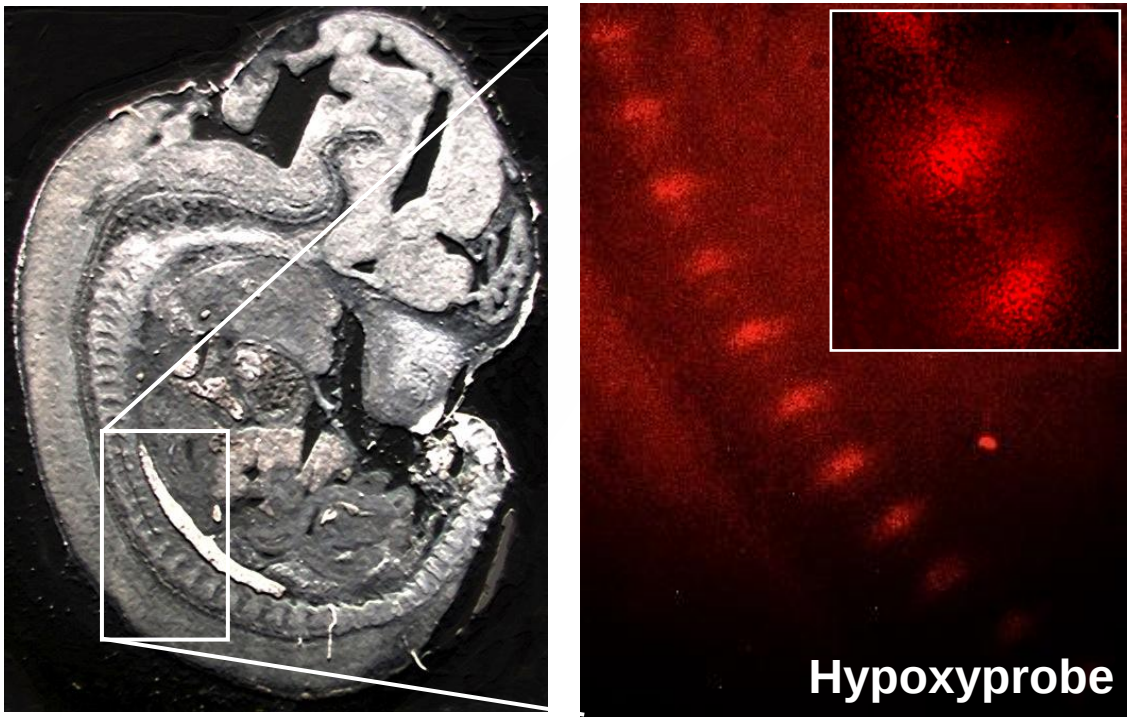
Endochondral bone development



Adapted from
Provot & Schipani. 2005. BBRC. 328: p.658

Physiological hypoxia

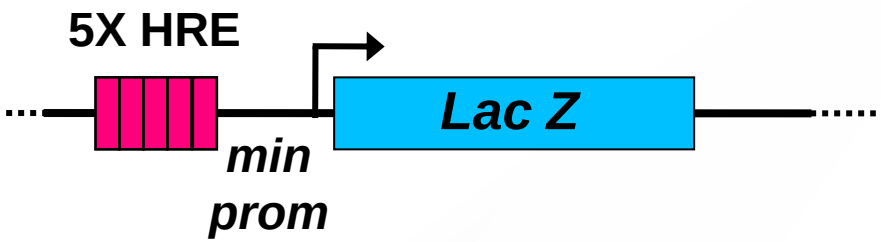
Role of Hif-1α in skeletal development

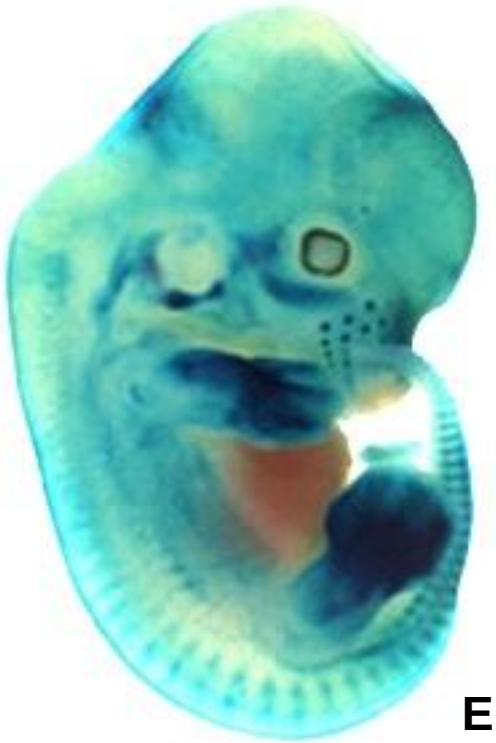


Mesenchymal condensations are hypoxic

Physiological hypoxia

Role of Hif-1α in skeletal development





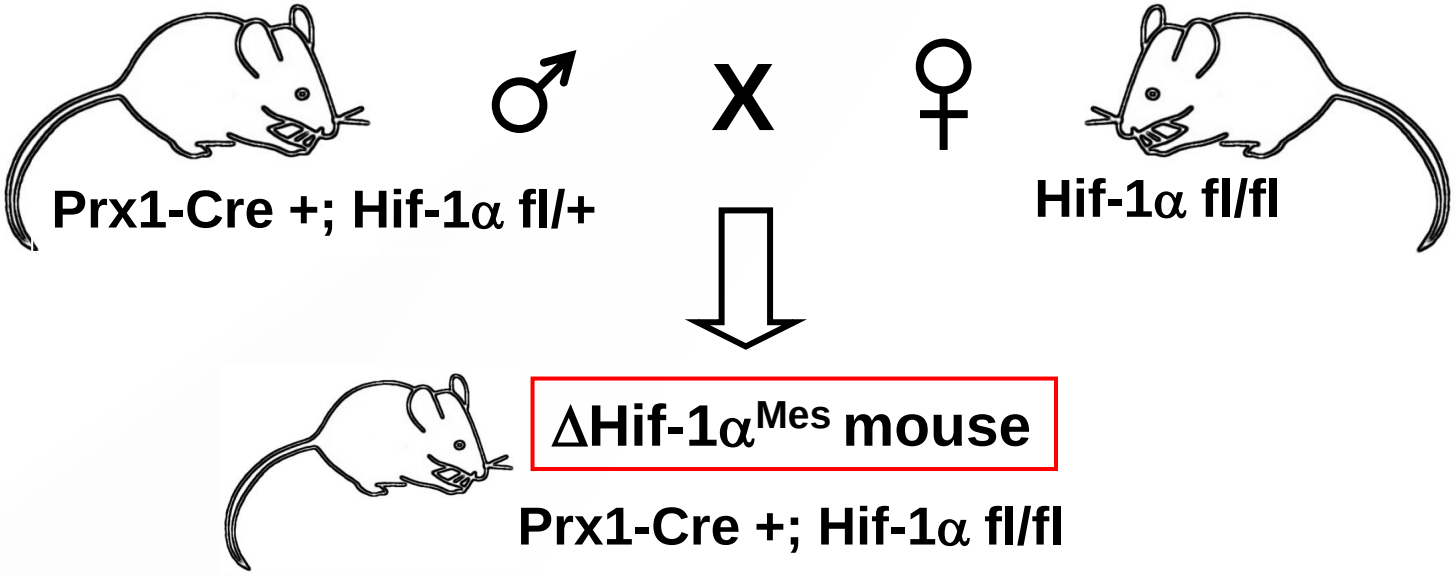
E12.5

Hif is transcriptionaly active in mesenchymal condensations

Physiological hypoxia

Role of Hif-1α in skeletal development

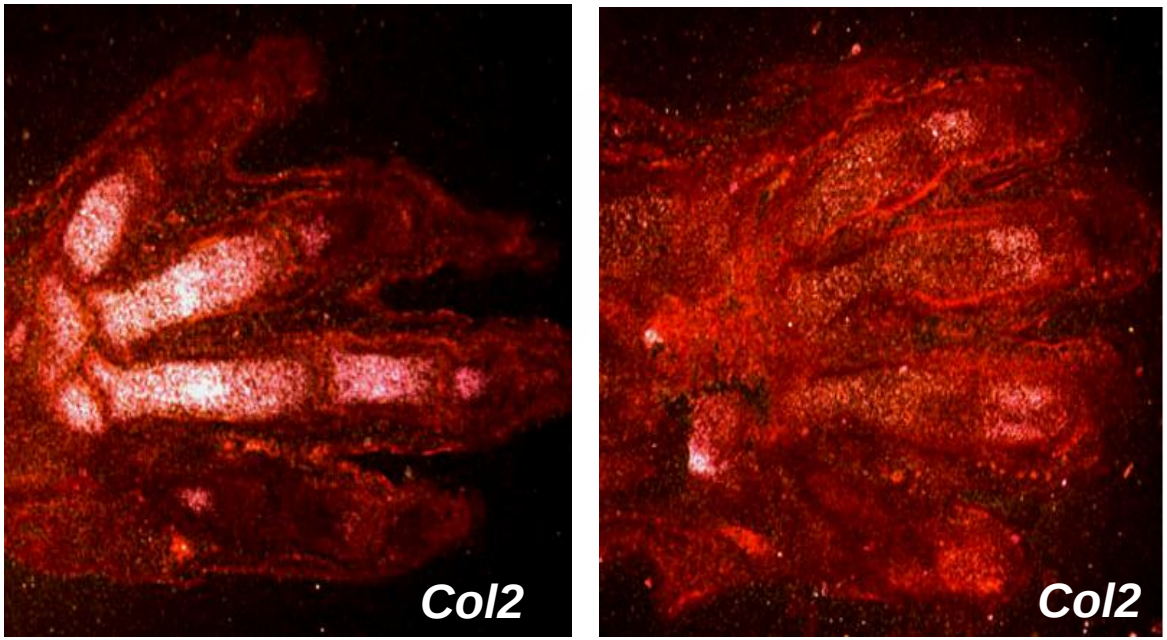
⇨ Removal of Hif-1α from the limb mesenchyme



Physiological hypoxia

Role of Hif-1α in skeletal development

Control **ΔHif1α^{mes}**



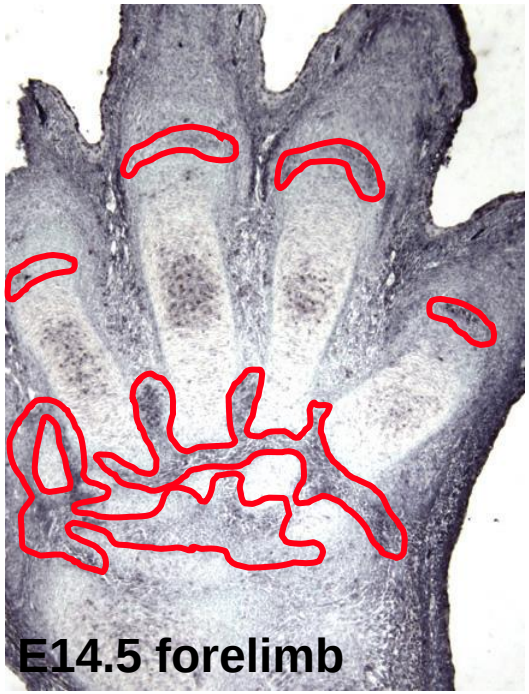
in situ hybridization

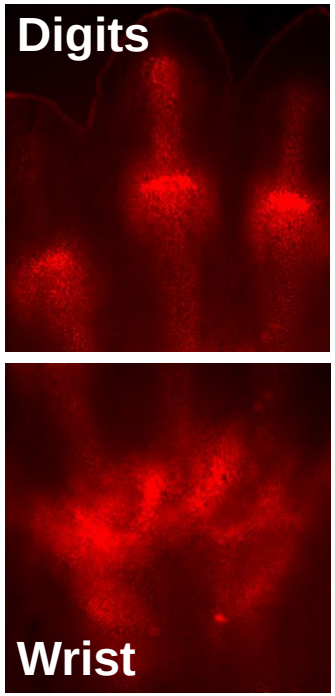
Hif-1α is required for chondrocyte differentiation

Physiological hypoxia

Role of Hif-1α in skeletal development

Hif1α protein





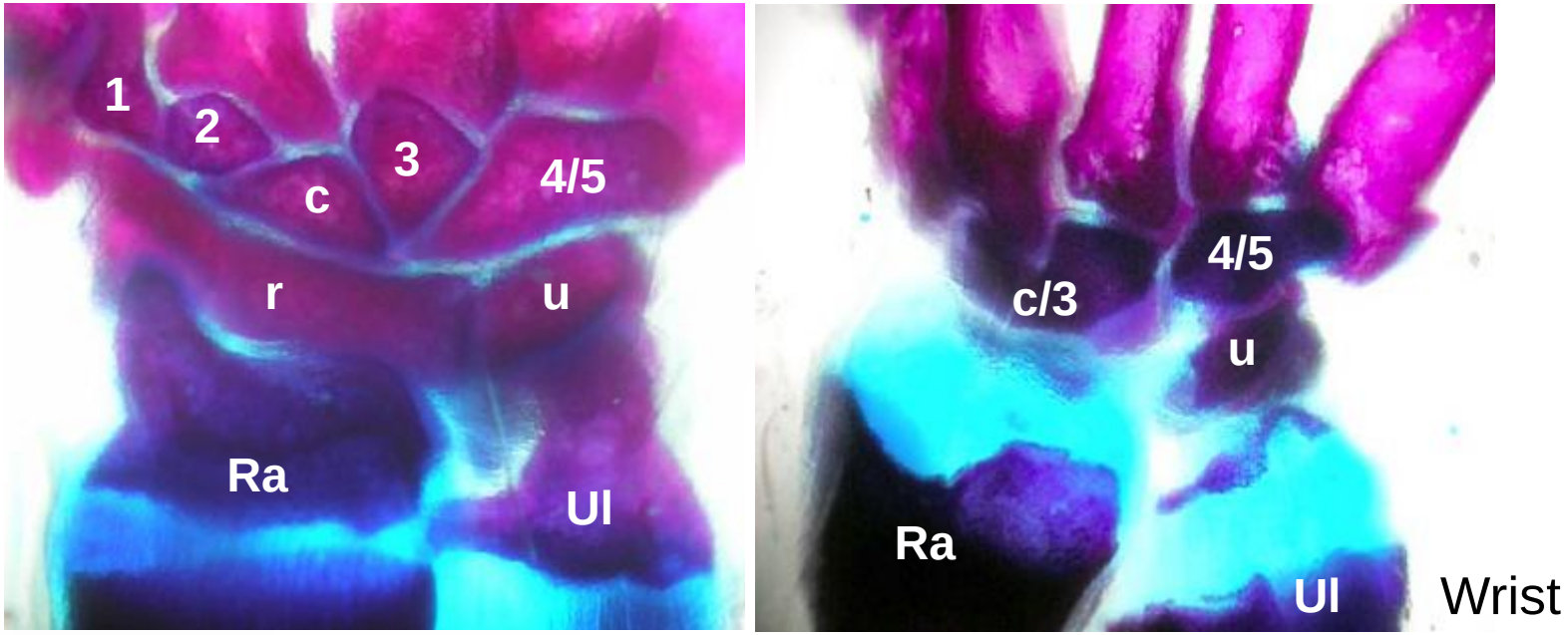
Hypoxyprobe

Developing joints are hypoxic and express Hif-1α

Physiological hypoxia

Role of Hif-1α in skeletal development

Control **ΔHif1α^{mes}**



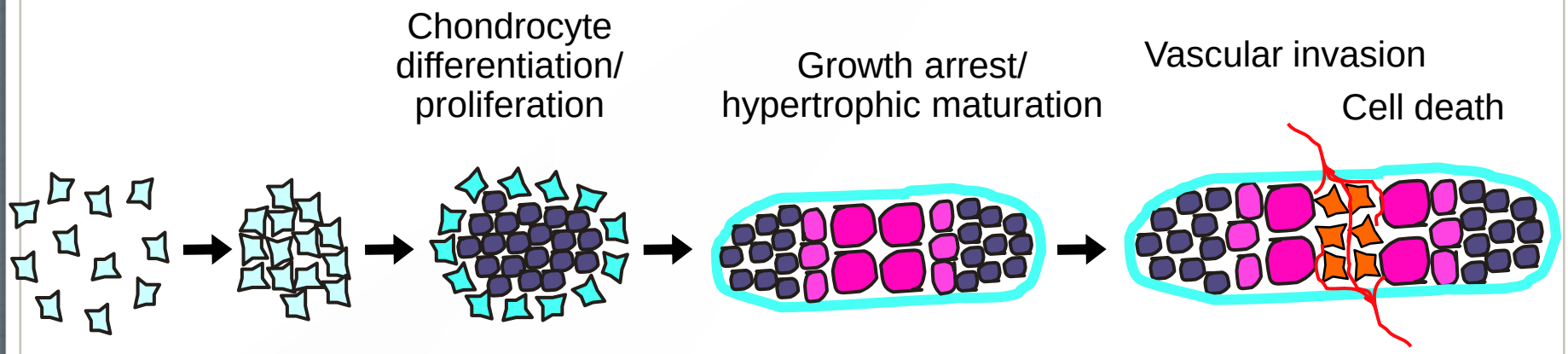
Wrist

Hif-1α is required for joint formation

Physiological hypoxia

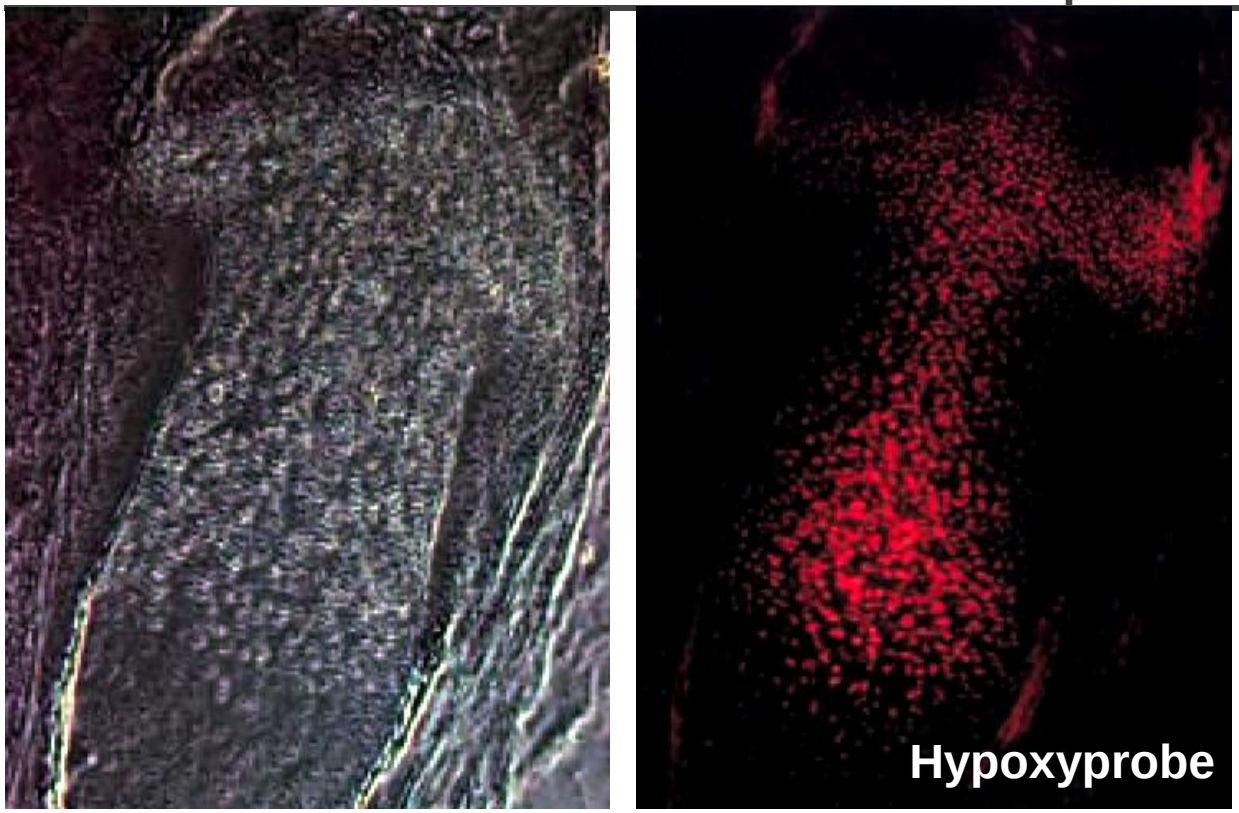
Role of Hif-1α in skeletal development

Role in growth plate chondrocytes?



Physiological hypoxia

Role of Hif-1α in skeletal development

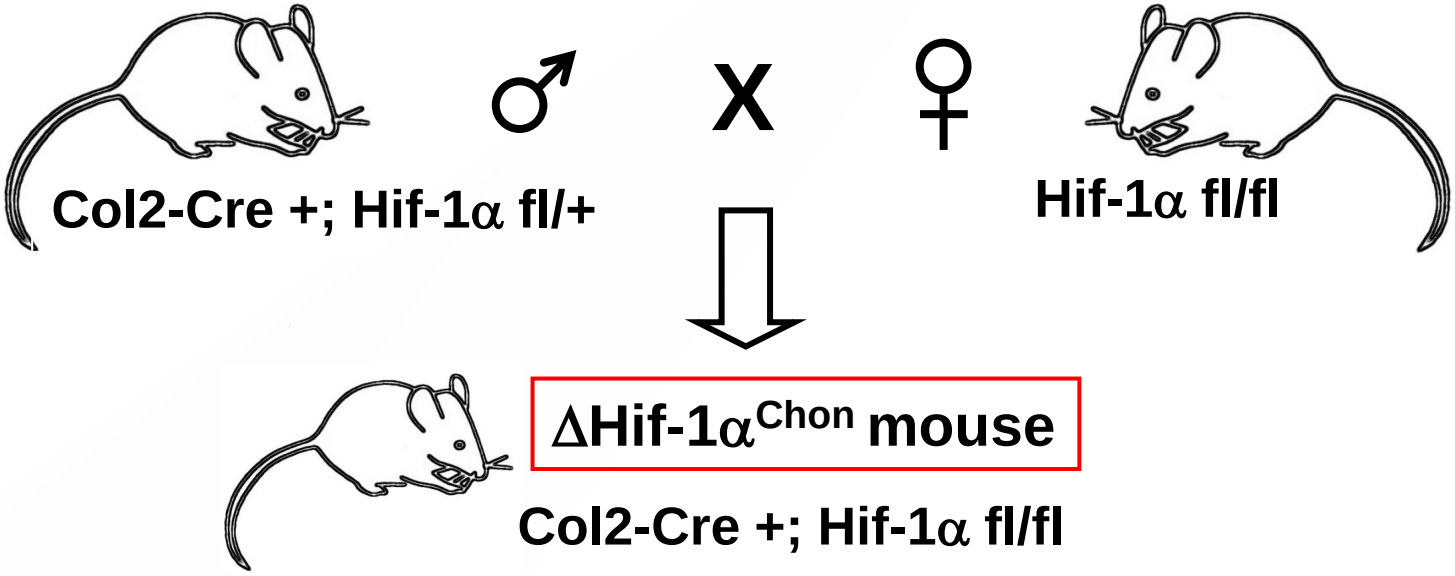


Growth plate cartilage is hypoxic and express Hif-1α

Physiological hypoxia

Role of Hif-1α in skeletal development

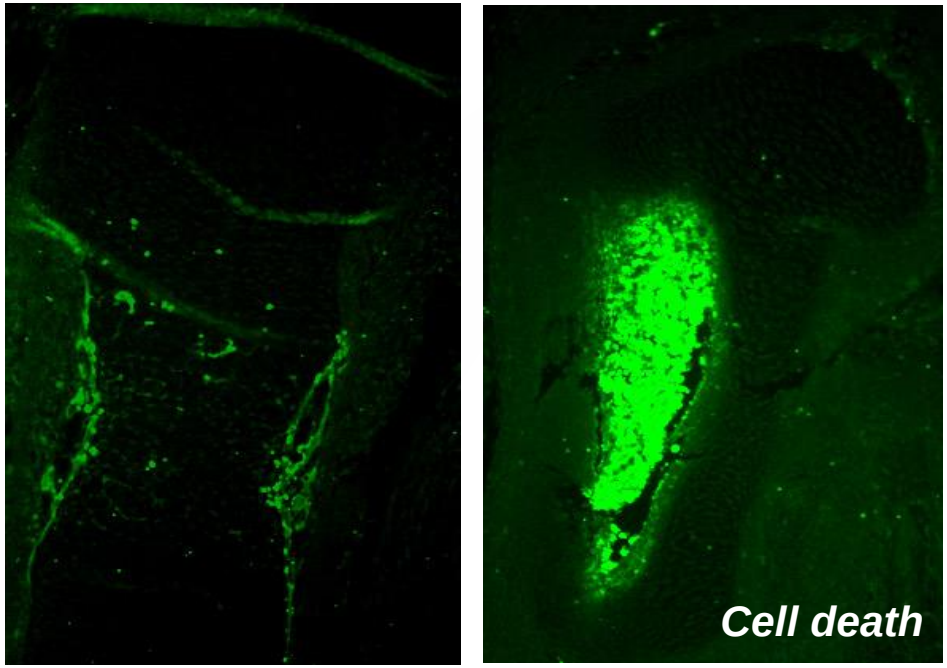
⇨ Removal of Hif-1α from chondrocytes



Physiological hypoxia

Role of Hif-1α in skeletal development

Control **ΔHif1α^{Chon}**



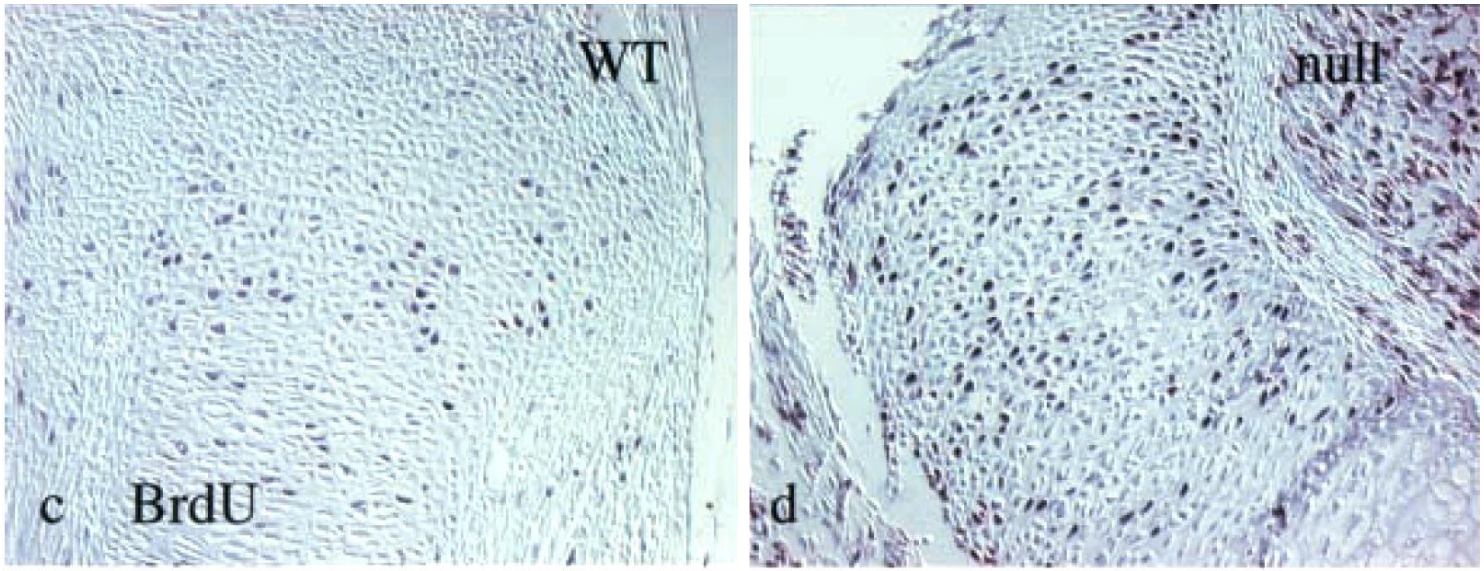
Tunel

Hif-1α is required for chondrocyte survival

Physiological hypoxia

Role of Hif-1α in skeletal development

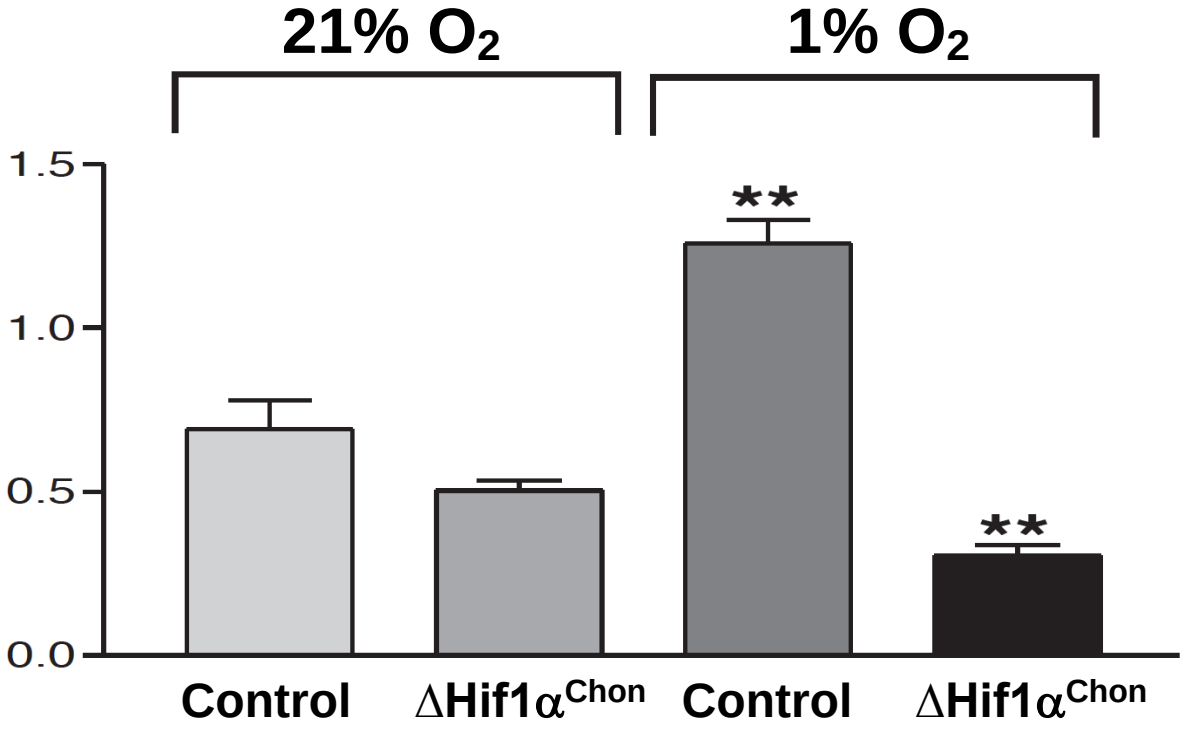
Control **ΔHif1α^{Chon}**



Hif-1α inhibits chondrocyte proliferation

Physiological hypoxia

Role of Hif-1α in skeletal development

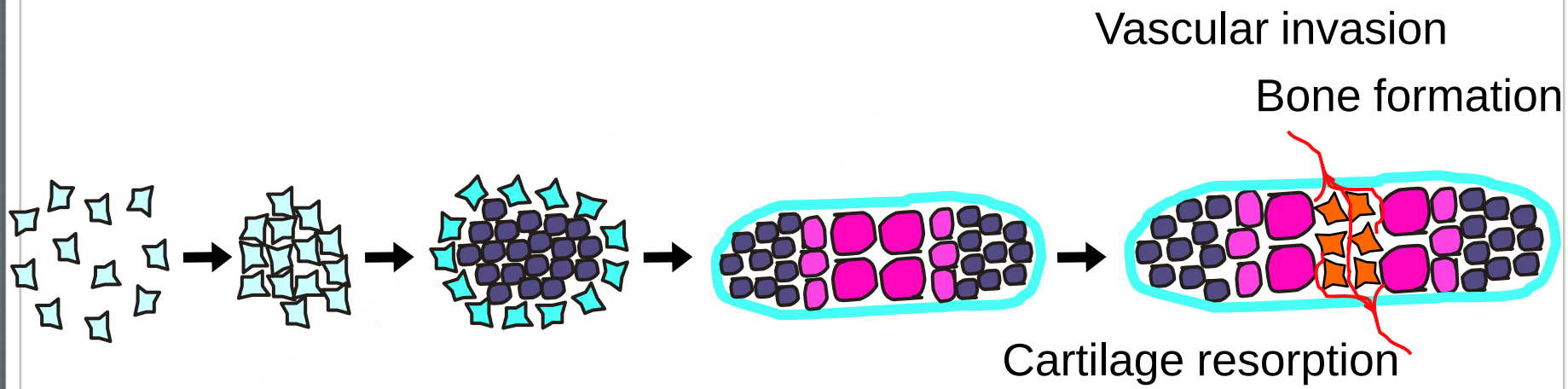


Hif-1α is required for cartilage matrix synthesis

Physiological hypoxia

Role of Hif-1α in skeletal development

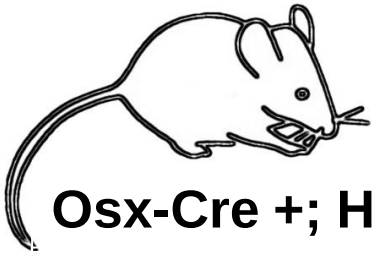
Role in bone formation?



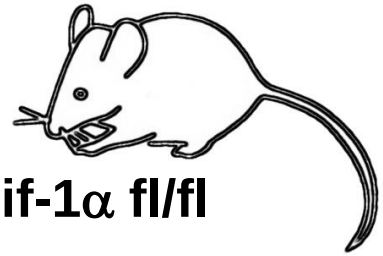
Physiological hypoxia

Role of Hif-1α in skeletal development

⇨ Removal of Hif-1α from osteoblasts



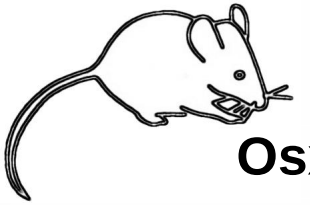
♂ **x** ♀



Osx-Cre +; Hif-1α fl/+
or OC-Cre +; Hif-1α fl/+

Hif-1α fl/fl

⇩

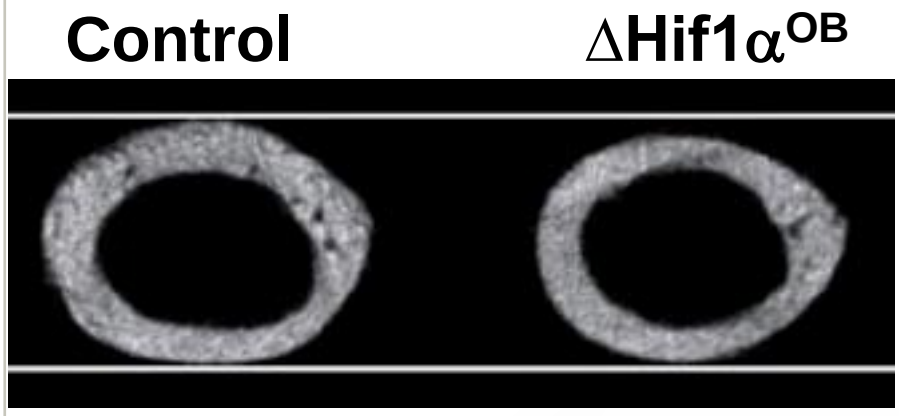


ΔHif-1α^{OB} mouse

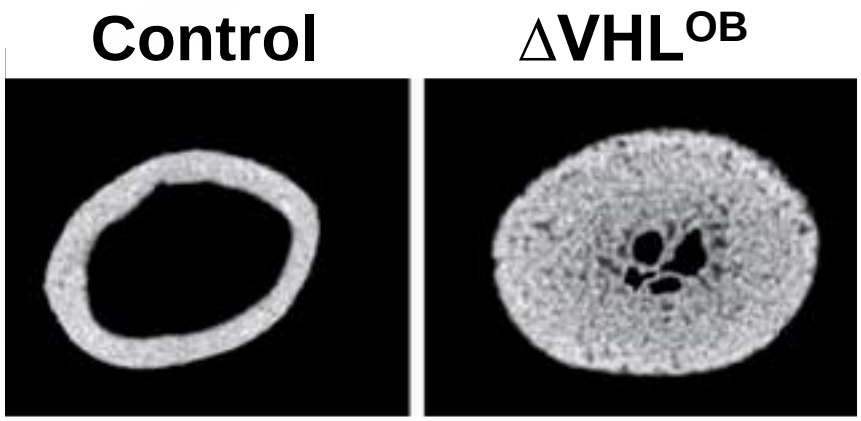
Osx-Cre +; Hif-1α fl/fl or OC-Cre +; Hif-1α fl/fl

Physiological hypoxia

Role of Hif-1α in skeletal development



↓ **Osteoblast number**

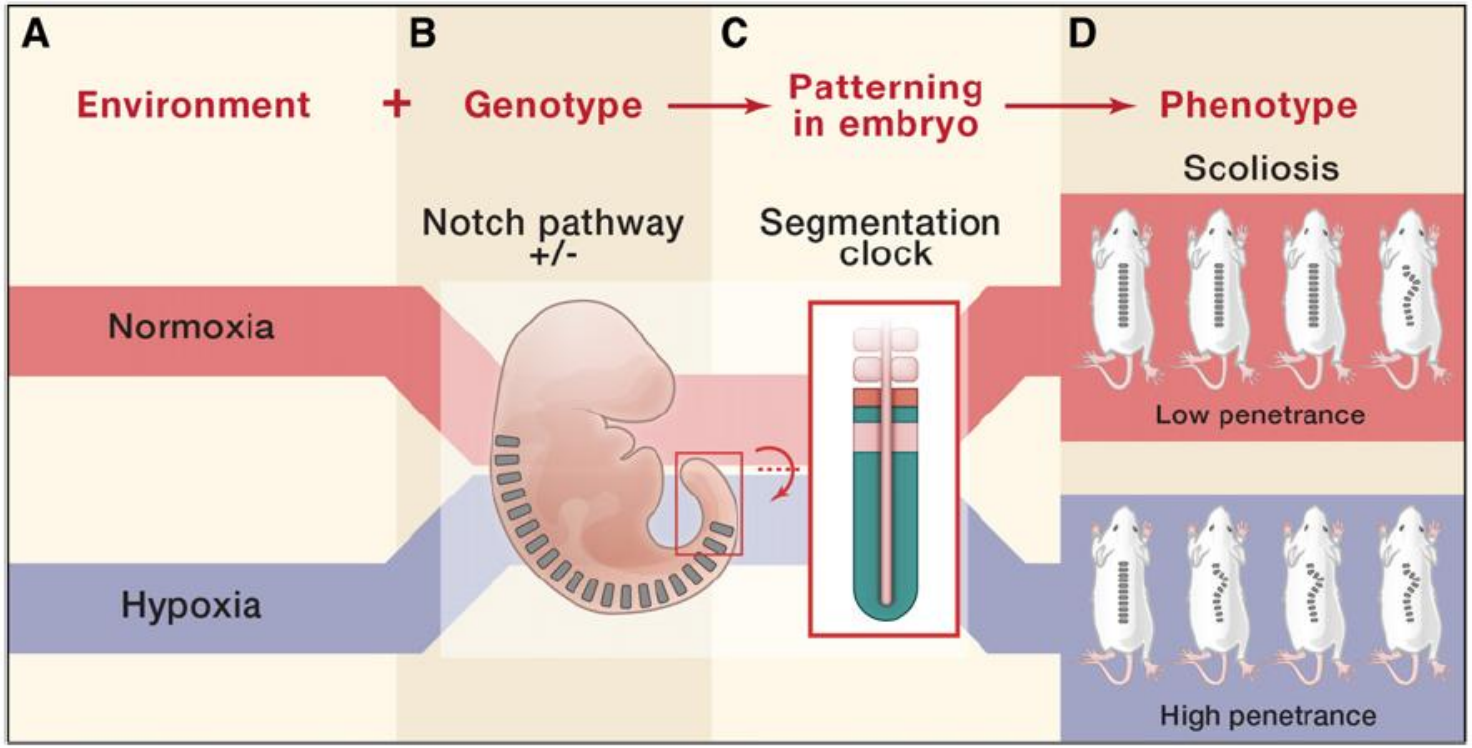


↑↑ **Osteoblast number**

Hif-1α is required for bone formation

Physiological hypoxia

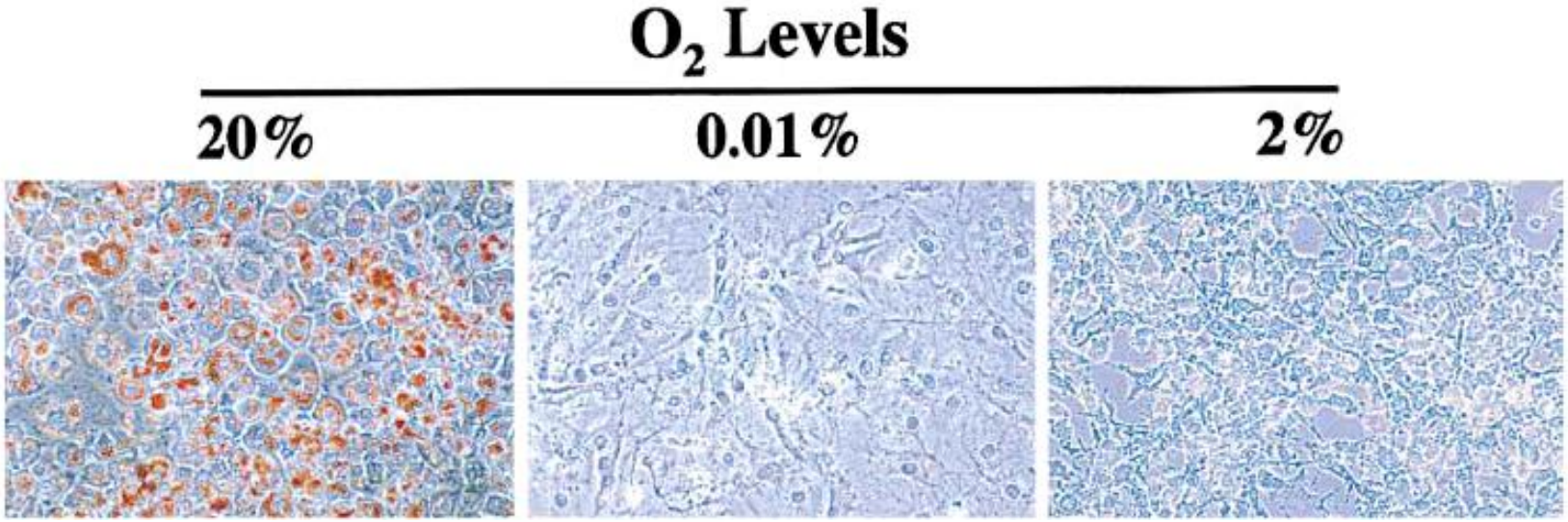
Role of HIF in congenital scoliosis



Hypoxia + Notch haploinsufficiency leads to scoliosis

Physiological hypoxia

Role of Hif-1α in adipogenesis



Oil Red O staining

Hif-1α mediates hypoxia-induced inhibition of adipogenesis in MEFs in vitro

Physiological hypoxia

Role of Hif-1α in the hematopoietic system

1- Lack of Hif-1α leads to abnormal B cell differentiation and autoimmunity

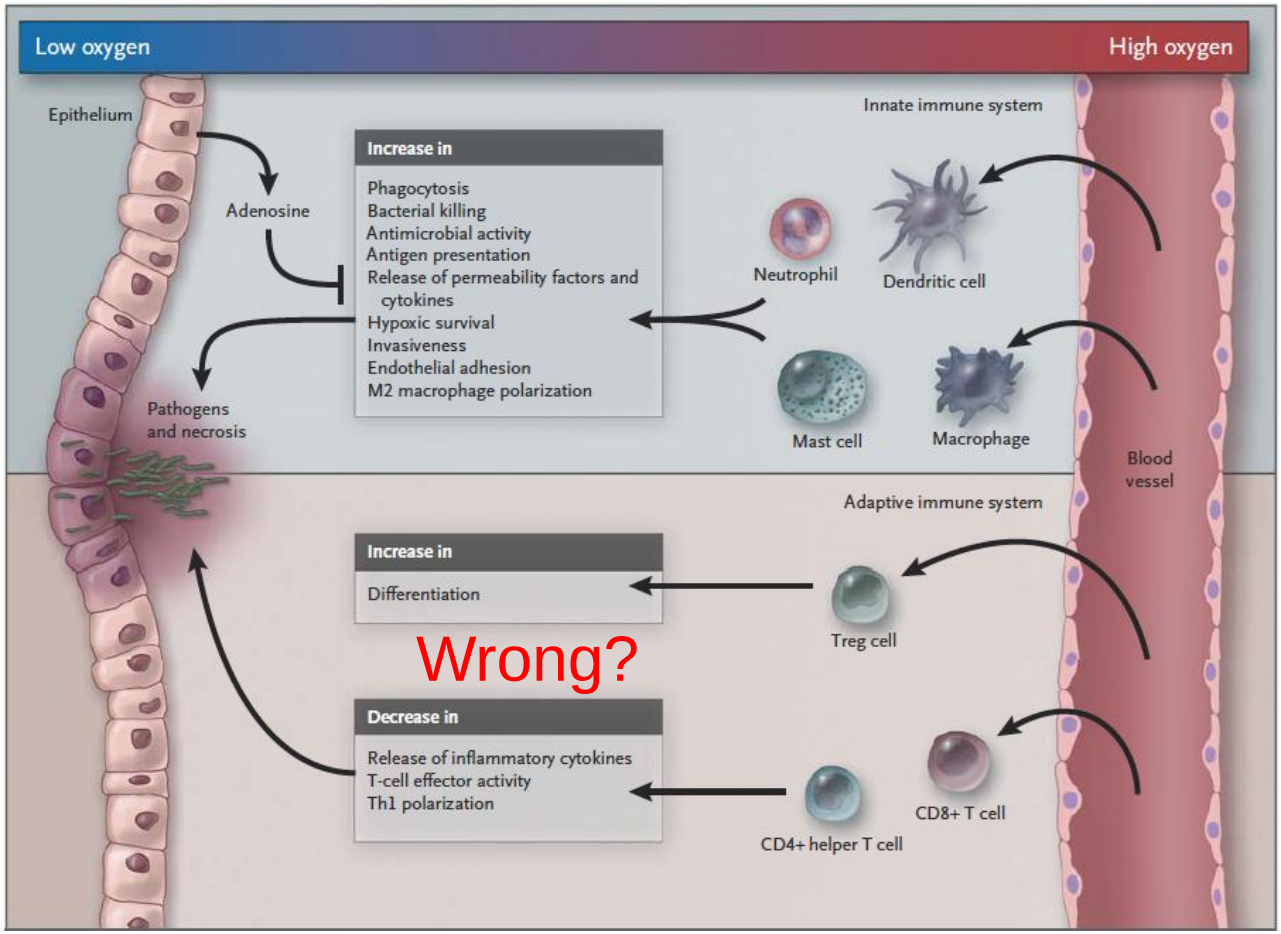
2- Proper Hif-1α protein level required to maintain cell cycle quiescence in HSC.

3- Hif-1α stimulates T_H17 and inhibits T_{reg} development.

4- Hif-1α stimulates the innate immune system.

Physiological hypoxia

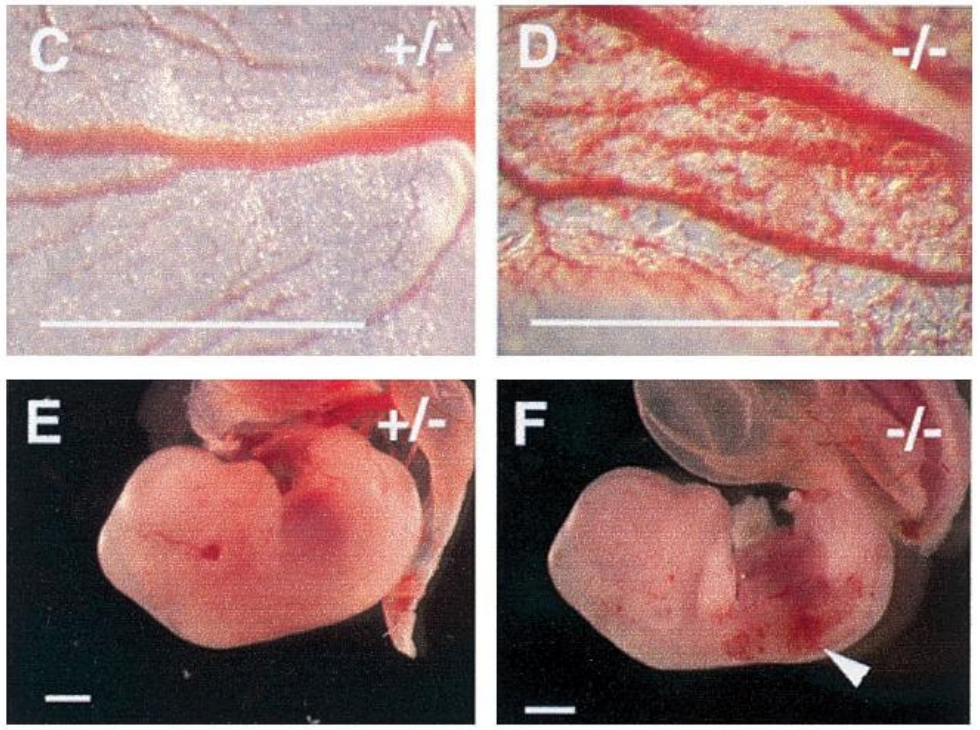
Role of Hif-1α in the hematopoietic system



Physiological hypoxia

Hif-2α knockout mice

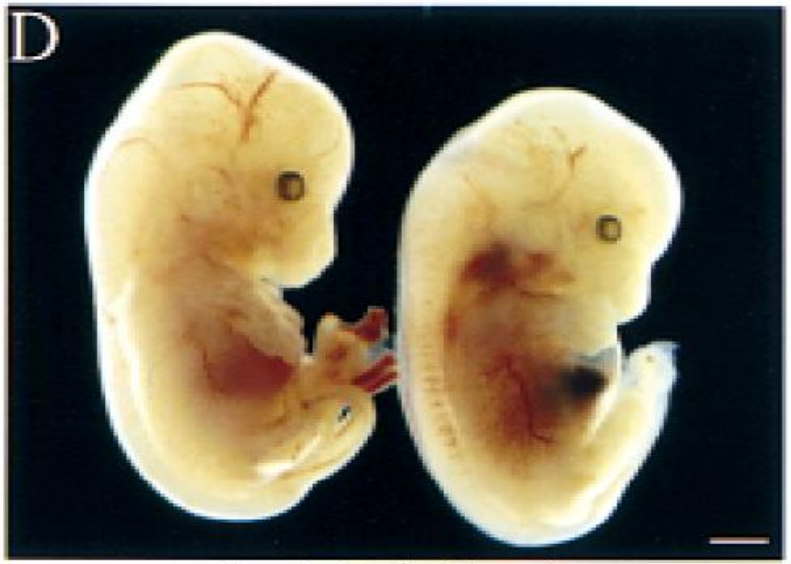
- Phenotype depends on genetic background
- Embryonic death at ED12.5 due to vascular defects (hemorrhages)



Physiological hypoxia

Hif-2α knockout mice

- Mid-gestation death du to bradycardia (no catecholamine) leading to heart failure



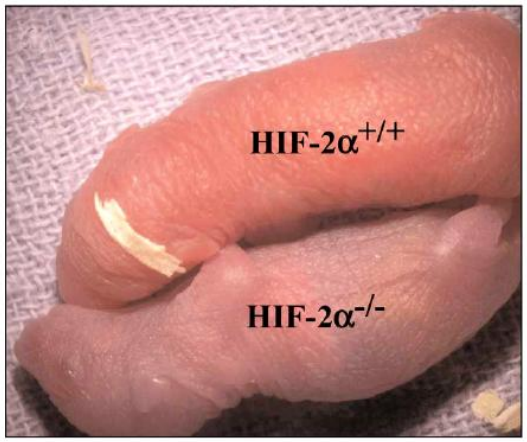
+/- E13.5 -/-

Physiological hypoxia

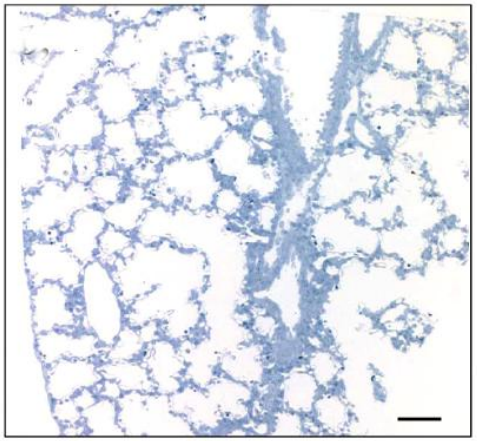
Hif-2α knockout mice

- Neonate death due to impaired lung maturation

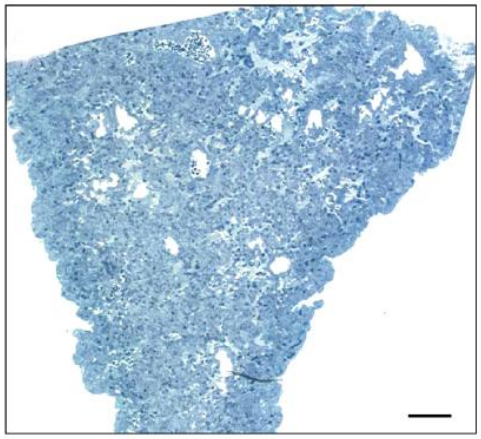
Cyanosis in KO mice



Lung histology



Control

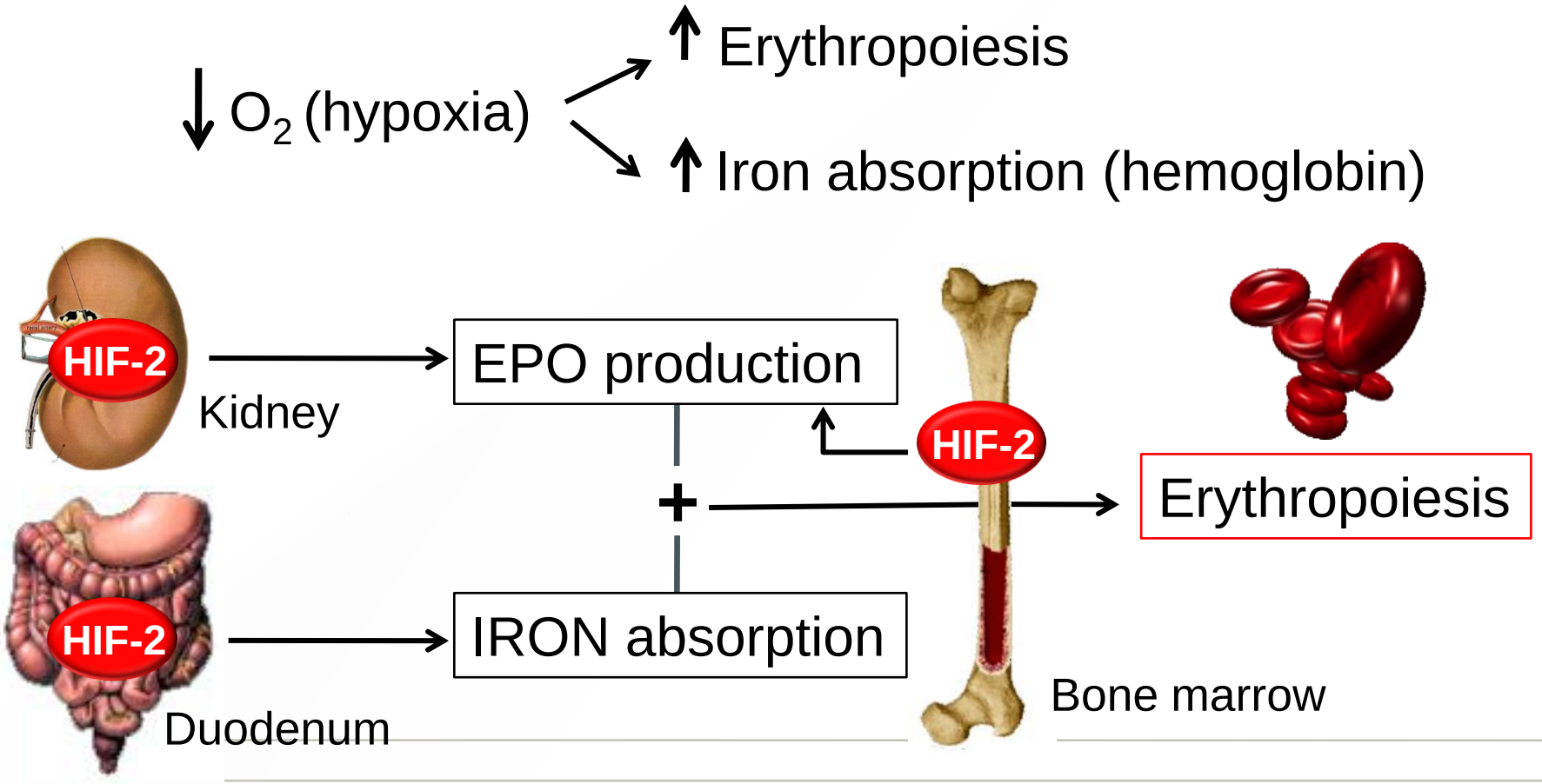


KO

- Adult death due to ROS-mediated multiorgan failure

Physiological hypoxia

Role of Hif-2α in iron metabolism & erythropoiesis



Pathological hypoxia

Protective responses mediated by HIF-1

- In coronary artery diseases: HIF-1 remodels collateral vessels around narrowed coronary artery to prevent myocardial infarction due to atherosclerotic plaques rupture
- Peripheral arterial disease: HIF-1 protects from limb ischemia (gangrene) by remodeling collateral blood vessels around arteries affected by atherosclerotic stenosis

Pathological hypoxia

Protective responses mediated by HIF-1

- Wound healing: HIF-1 mediates angiogenesis (budding of new capillaries from existing vessels), and vasculogenesis (de novo blood vessel formation) by secreting angiogenic factors and cytokines such as SDF1, which permits the recruitment of BMDAC in the wound.
- Organ transplant rejection: destruction of microvasculature of the tissue engrafted leads to chronic rejection. HIF-1-dependent recruitment of recipient Tie2+ angiogenic cells repairs microvasculature and allows graft survival.

Pathological hypoxia

Increased pathological risks mediated by HIF-1

- Cancer:

  - The von Hippel Lindau syndrome: VHL loss of function mutations lead to pheochromocytomas, and highly vascularized tumors of the kidney, the central nervous, and the retina.

  - HIF-1 stimulates hypoxic tumor growth (angiogenesis, metabolism, etc.), and dissemination (LOX induction promotes metastatic niches).

Pathological hypoxia

Increased pathological risks mediated by HIF

- Traumatic shock:

  Abdominal trauma/hemorrhagic shock leading to severe pulmonary inflammatory response and intestinal villous necrosis is attenuated in absence of HIF-1α in mice.

- Pulmonary arterial hypertension:

  Chronic lung disease leads to prolonged alveolar hypoxia which causes increased pulmonary arterial pressure, and right ventricular hypertrophy that are attenuated in HIF-1α and HIF-2α CKO mice.

Pathological hypoxia

Increased pathological risks mediated by HIF

- Obstructive sleep apnea:

  - Pharyngeal soft tissue occludes the airway periodically, causing dozens of hypoxic cycles.

  - This leads to increased amounts of ROS in the carotid body and the brain, leading to increased catecholamine levels, and systemic hypertension.

  - This phenomenon is mediated by HIF-1α.

“Take-home” messages

- O_2= signaling molecule
- Normoxia ≠ 21% O_2 in all living organisms
- Hypoxia ➔ Oxydative-to-glycolytic metabolism switch
- No HIF1 ➔ ↓↓ ATP + ↑↑ROS ➔ Cell death
- HIF1 = Survival factor **AND** differentiation factor

“Take-home” messages

- HIF signaling: VHL ⊣ Hif-1α → VEGF + other genes
- Hypoxia/HIF plays critical in many physiological and pathological mechanisms ➔ A future Nobel Price?
- There are HIF-independent responses to hypoxic stress: mTOR signaling and the Unfolded Protein Response (UPR) regulate protein synthesis, metabolism, and cell fate in response to hypoxia.

Acknowledgements

- The key bibliographic references:

  Semenza GL. (2012). Hypoxia-Inducible Factors in Physiology and Medicine. Cell 148, Feb. 3, pp. 399-408.

  Majmundar AJ., Wong WJ., and Celeste Simon M. (2010). Hypoxia-Inducible Factors and the Response to Hypoxic Stress. Molecular Cell 40, Oct. 22, pp. 294-309.

- Collaborators:

  Stina Schipani, MD, PhD, Indiana University, USA.

  Carole Peyssonnaux, PhD, Institut Cochin, Paris.

  Jay Kim, PhD, UC San Diego, USA.

To contact me: sylvain.provot@inserm.fr

Drugs “controlling” HIF

Table 1. Drugs that Inhibit HIF-1

Process Inhibited	Drug Class	Prototype
HIF-1α protein synthesis	cardiac glycoside	digoxin
	mTOR inhibitor	rapamycin
	microtubule-targeting agent	2-methoxyestradiol
	topoisomerase I inhibitor	topotecan
HIF-1α protein stability	HDAC inhibitor	LAQ824
	HSP90 inhibitor	17-AAG
	calcineurin inhibitor	cyclosporine
	guanylate cyclase activator	YC-1
Heterodimerization	antimicrobial agent	acriflavine
DNA binding	anthracycline	doxorubicin
	quinoxaline antibiotic	echinomycin
Transactivation	proteasome inhibitor	bortezomib
	antifungal agent	amphotericin B
Signal transduction	BCR-ABL inhibitor	imatinib
	cyclooxygenase inhibitor	ibuprofen
	EGFR inhibitor	erlotinib, gefitinib
	HER2 inhibitor	trastuzumab

Drugs activating HIF-1

Dimethyloxalylglycine (DMOG) :
inhibits hydroxylases and induces HIF-1-dependent transcription

Desferrioxamine and Cobalt Chloride: iron chelators that inhibit hydroxylases by displacing Fe(II) from the catalytic center

Measuring tissue O_2 tension (PO_2) experimentally

- Polarographic method: probe permeable to oxygen filled with electrolytes and two electrodes measuring an electrical potential resulting from an O_2-dependent chemical reaction.
- OxyLite PO_2 system (Oxford Optronics): measure of PO_2 using a fluorescence quenching technique.
- The Hypoxyprobe (pimonidazole hydrochloride): Pimonidazole is reductively activated in hypoxic cells. The activated intermediate forms stable covalent adducts with thiol groups in proteins. An Ab is used to detect these adducts by IHC.
- Normal PO_2 range in tissues: 6-34 mm Hg (1-5%). (Atmosphere: 21% O_2 = 160 mm Hg; Arterial blood: 95 mm Hg)