Intracellular (nuclear and cytosolic) receptors

Ligand-dependent or environment-sensitive transcription factors

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Cell-surface vs. intracellular receptors
Cellular responses through cell-surface receptors

- Extracellular signal molecule
- Cell-surface receptor protein
- Intracellular signaling pathway
- Nuclear signaling pathway
- DNA
- RNA
- Altered protein synthesis
- Altered cytoplasmic machinery
- Altered cell behavior

**Timeframes:**
- FAST (< sec to mins)
- SLOW (mins to hrs)
Transcription factors controlled by extrinsic and intrinsic signals
Long-term cellular responses through intracellular receptors

[Diagram]

- **Inactive Receptor**
  - Transcription-activating domain
  - DNA-binding domain
  - Inhibitory proteins

- **Active Receptor**
  - Ligand-binding domain
  - Coactivator proteins
  - Ligand
  - Receptor-binding element
  - Transcription of target genes

DNA
Long-term cellular responses through intracellular receptors (often in two waves)
Main protein motifs involved in DNA binding
bHLH motif
SREBP sensing low cholesterol level

Induction of HMG-CoA reductase and LDL receptor

Increasing cholesterol level
bHLH/PAS transcription factors: (neurogenesis, circadian rhythm) and sensing xenobiotics or hypoxia

AHR: aromatic hydrocarbon receptor
HIF: hypoxia inducible factor
PAH: polyaromatic hydrocarbon

methylcholanthrene (MC)

benzo[a]pyrene (BaP)

HPAH: halogenated PAH

The biotransformation enzymes often fail to metabolize them

TCDD (dioxin)

TCDF

tetrachlorobiphenyl (PCB)
Ligand activation of AHR and control by negative feedback mechanisms

DRE: dioxin response element = XRE: xenobiotic response element
HIF-1
Hypoxia inducible factor
HIFs belong to a family of environmental sensors known as bHLH–PAS (basic helix-loop-helix–Per-Arnt-Sim) transcription factors.

Ubiquitous $\text{O}_2$-sensitive

In some tissues only $\text{O}_2$-sensitive

Ubiquitous $\text{O}_2$-insensitive

**HIF-1 ($\alpha \beta$ heterodimer)** binds to hypoxia response element (HRE) sequences in DNA.

TAD: transactivation domain

ODD: oxygen-dependent degradation domain
Regulated HIF-1 protein interactions

**ODD** = oxygen-dependent degradation domain

**VHL** = von Hippel-Lindau protein, E3 ligase

**TAD** = trans activation domain

**P300/CBP** = coactivator protein of HAT activity
Pro (and Asn) hydroxylation of HIF-1α are dependent on $P_{O_2}$
Pro (and Asn) hydroxylations of HIF-1α are dependent on $P_{O_2}$
Pro (and Asn) hydroxylations of HIF-1α are dependent on $P_\text{O}_2$

Active HIF hydroxylases

- PHDs
- FIH

Unstable HIFα

HO-Pro Asn-OH

- pVHL-mediated proteolysis
- Blocked p300 co-activator recruitment

Inactivation of HIF transcriptional activity

Inactive HIF hydroxylases

- Stable HIFα

Pro Asn

- Co-activator recruitment

p300

Activation of HIF transcriptional activity

**FIH** (factor inhibiting HIF) Asn hydroxylase

**PHDs** (prolyl hydroxylase domain proteins) Pro hydroxylases
HIF-1 response

Hypoxia → MAP kinase PKB → Stabilized HIF1 dimer → HIF1 target gene promoter

MAP kinase PKB

Stabilized HIF1 dimer

HIF1 target gene promoter

Transcriptional activation

iNOS → NO → Angiogenic → Improved blood flow → Improved cell survival

VEGF, EPO → Angiogenic

GLUT1 → Glucose → Lactate → ATP → Improved cell survival

Glycolytic enzymes → Glucose → Lactate → ATP → Improved cell survival

Others → ATP → Improved cell survival
HIF-1 response

HIF-1 transcription factor controls:

• Glucose uptake
• Glycolysis (and lactate production)
• Angiogenesis
• Oxygen delivery – erythropoiesis
• Growth and survival
• Iron metabolism
• pH homeostasis
• Autophagy
HIF-1 response

**Glucose transporters**
- GLUT-1
- GLUT-3

**Glycolysis and other (energy transforming) enzymes**
- Adenyl kinase 3
- Aldolase A, C
- Enolase-1
- Phosphofructokinase L
- Phosphoglycerate kinase1
- Glycerinaldehyde-3-P dehydrogenase
- Heme oxygenase-1
- Hexokinase 1,2
- Carbon anhydrase 9
- Lactate dehydrogenase
- NO synthase 2
- Prolyl-4-hydroxylase α(I)
- Pyruvate kinase M
- Triose-phosphate isomerase

**Growth, survival, and angiogenic factors**
- Cyclin G2
- Endothelin-1
- Erythropoetin
- Insulin like growth factor 2 (IGF2)
- Transforming growth factor (TGF)
- VEGF
- VEGF receptor FLT-1

**Other proteins**
- Coeruloplasmin
- Collagen (type V,α1)
- P-glycoprotein
- Plazminogen activator inhibitor 1
- Transferrin
- Transferrin receptor
Pathogenic role of hypoxia and HIF

- Ischaemia diseases – infarction, stroke
- Chronic obstructive pulmonary diseases
- Malignant tumors
- Atherosclerosis
- Diabetes mellitus
- Inflammatory diseases
- Psoriasis
- Pre-eclampsia
Aerobic ATP production in normoxia

extracellular space → GLUT1 → glycolysis → pyruvate

- 2ATP
- biosynthesis
- lactate

cytosol → PIP3 → PI3K → Ras → PKB/Akt → Myc

normal cells → HIF1 → hypoxia

mitochondrion → PDH → acetyl CoA

Krebs/citric acid cycle

O₂ → CO₂

~32 ATP
Anaerobic ATP production in hypoxia

cancer cells

genes/proteins hyperactive in many cancer cells

hypoxia

Krebs/citric acid cycle

O₂ → CO₂

~32 ATP

extracellular space

cytosol

biosynthesis

lactate

mitochondrion

2ATP

PK-M2

LDH

~4ATP

acetyl CoA

pyruvate

PKB/Akt

HIF1

PI3K

Ras

PIP3

Myc

glucose → GLUT1 → glycolysis → biosynthesis