Redox Homeostasis and Radical Detoxification Systems in Mycobacterium tuberculosis

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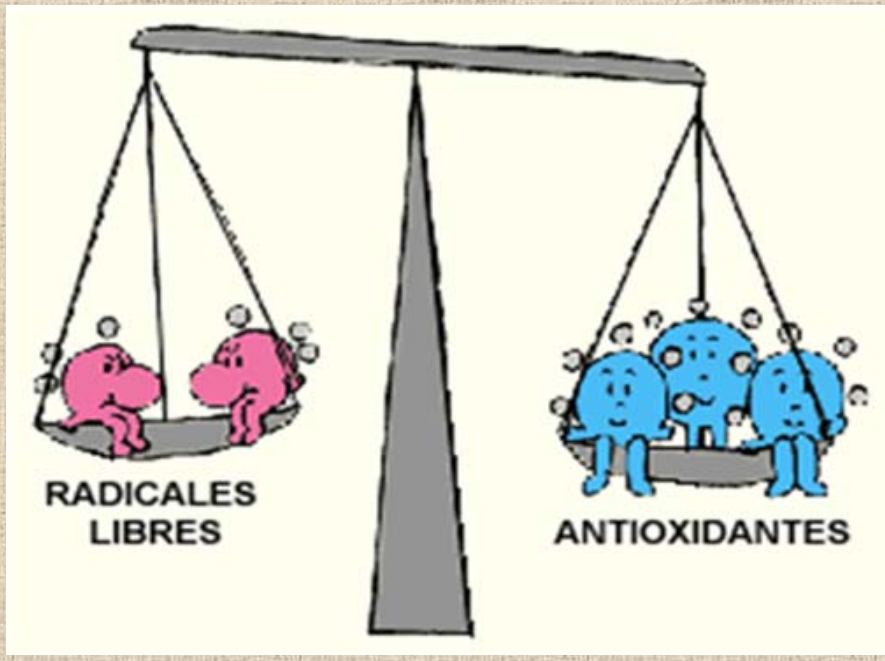
Outline

- Redox Homeostasis & Mtb
- Oxidative Stress in *Mtb* lifecycle
  I. ROS
  II. RNS
- Redox Homeostasis in *Mtb*
  I. redox couples (buffer)
  II. enzymes
- Redox sensing in *Mtb*
  I. stringent response
  II. DosR regulation system
  III. WhiB proteins as sensor
- Summary

Part I. Redox Homeostasis & *Mycobacterium tuberculosis*

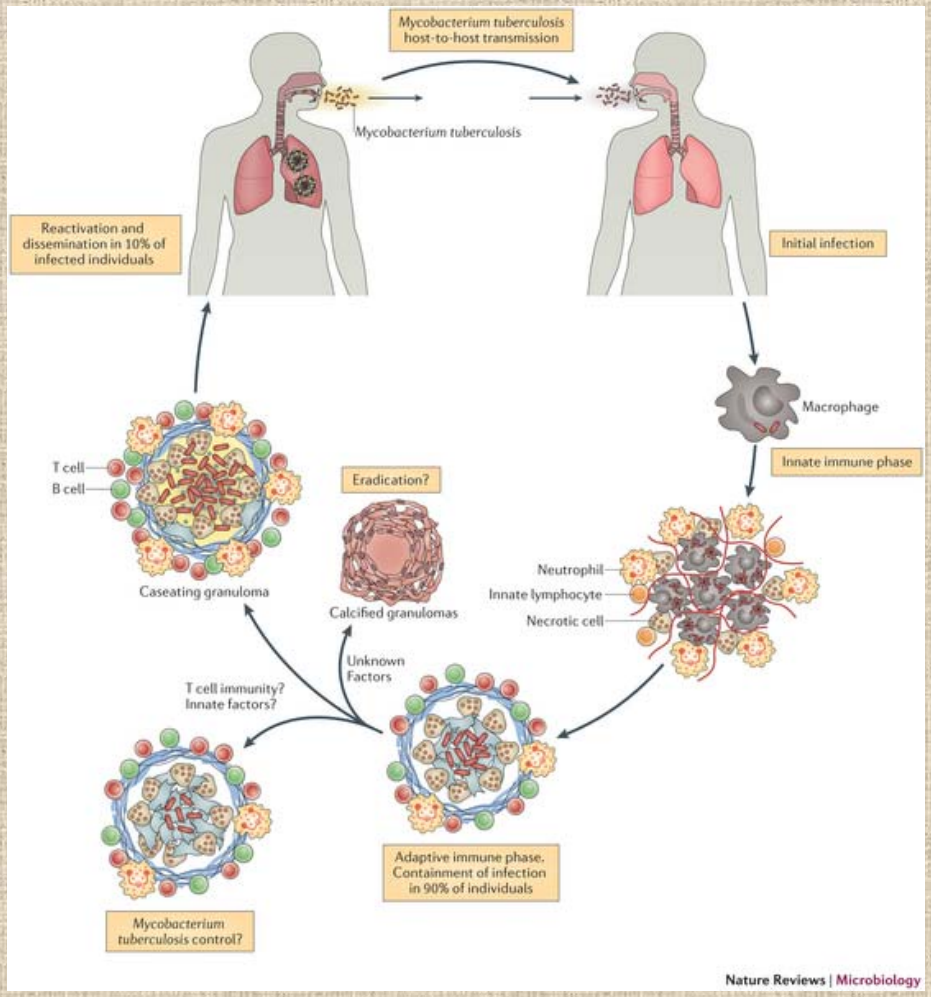
Redox Homeostasis

- The balance of oxidative and reductive capacity within a biological system such as a single cell, organ, or organism
- The reactive species will produce in all aerobic respiration
- Oxidative stress: Reactive oxygen species(ROS) e.g. $O2^{\bullet}$-, $HO2^{\bullet}$, $HO^{\bullet}$ and $RO^{\bullet}$; Reactive oxygen species; Reactive nitrogen species (RNS) e.g. $NO^{\bullet}$, $NO2^{\bullet}$ and $NO3^{\bullet}$
- Antioxidant defense: Enzymatic; Non-enzymatic



Extra difficulty for *Mtb* Redox Homeostasis

- As a pathogen, need to evade most **immune stress** from host cell
- **ROS/RNS** is most significant immune stress in macrophage
- Redox imbalance might also affect **antimycobacterial drug efficacy**. For example, INH or ethionamide



A typical infection of Mtb

"captured→survive in macrophage (→ enter dormancy → → resuscitation) → active infection"

Standard redox potential of normal redox stress species

- Radical species damage microbial DNA, lipids, and proteins, as well as other susceptible cellular constituents.
- The higher of Redox potential, the higher ability to make damage

Table 1. Standard reduction potentials of biologically relevant redox couples

Redox couple	Redox potential (mV)
$CO_2/CO_2^{\bullet-}$	−1800
CO_2/CO	−520
Acetyl-Co/Apyruvate	−500
Succinyl-CoA/2-oxoglutarate	−491
$CO_2/HCOO^-$	−421
H^+/H_2	−414
$NAD^+/NADH$	−316
$NADP^+/NADPH$	−315
CO_2/acetate	−291
TrxC [TrxSS/Trx(SH_2)]	−269
TrxB [TrxSS/Trx(SH_2)]	−262
TrxA [TrxSS/Trx(SH_2)]	−248
$2H^+$/2Cys-SH (cystine)	−230
$FAD^+/FADH_2$	−219
$FMN^+/FMNH_2$	−219
Pyruvate, H^+/lactate	−183
Oxaloacetate, $2H^+$/malate	−166
Menaquinone	−74
ESSE/2ESH (ergothioneine)	−60
$CoQ/CoQ^{\bullet-}$	−36
Fumarate/succinate	+32
Ubiquinone/ubiquinol	+45
Fe^{3+}/Fe^{2+}(aq)	+110
$Ascorbate^{\bullet-}$/ascorbate$^-$	+282
O_2/H_2O_2	+295
Cytochrome a_3 (Fe^{3+})/cytochrome a_3 (Fe^{2+})	+350
NO_3^-/NO_2^-	+421
α-Tocopheroxyl$^{\bullet}$/α-tocopherol	+500
O_2/H_2O	+818
$RS^{\bullet}/RS^-$ (cysteine)	+920
$GS^{\bullet}/GS^-$ (glutathione)	+920
$NO_2^{\bullet}/NO_2^-$	+990
$ROO^{\bullet}$, H^+/ROOH (alkyl peroxyl radical)	+1000
$HO_2^{\bullet}$, H^+/H_2O_2	+1060
$ONOO^-/NO_2^{\bullet}$ (aq)	+1400
$RO^{\bullet}$, H^+/ROH (aliphatic alkoxyl radical)	+1600
$NO_2^-/NO_2^{\bullet}$	+1600
$CO_3^{\bullet-}$, H^+/HCO_3^-	+1780
$HO^{\bullet}$, H^+/H_2O	+2310

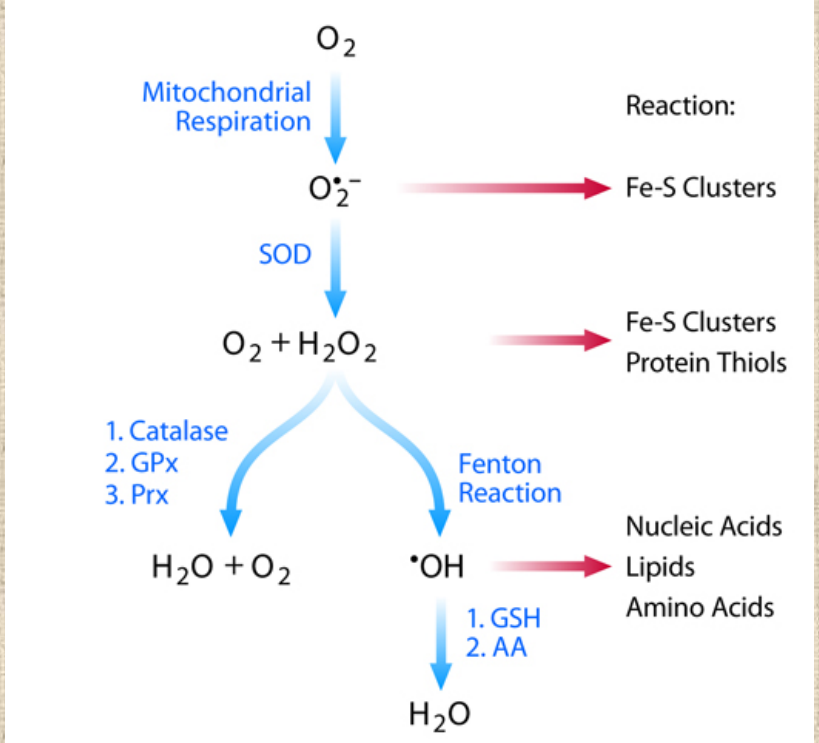
Highly reducing

Highly oxidizing

Part II. Redox Stress in *Mtb* lifecycle

Endogenous ROS stress

- reduction of O2 by various components of the electron transport chain under normal aerobic conditions, resulting in the production of ROS as superoxide radicals (O2•-).
- O2•- also oxidises the 4Fe–4S clusters of enzymes, such as dehydratases, leading to enzyme inactivation and release of Fe2+.



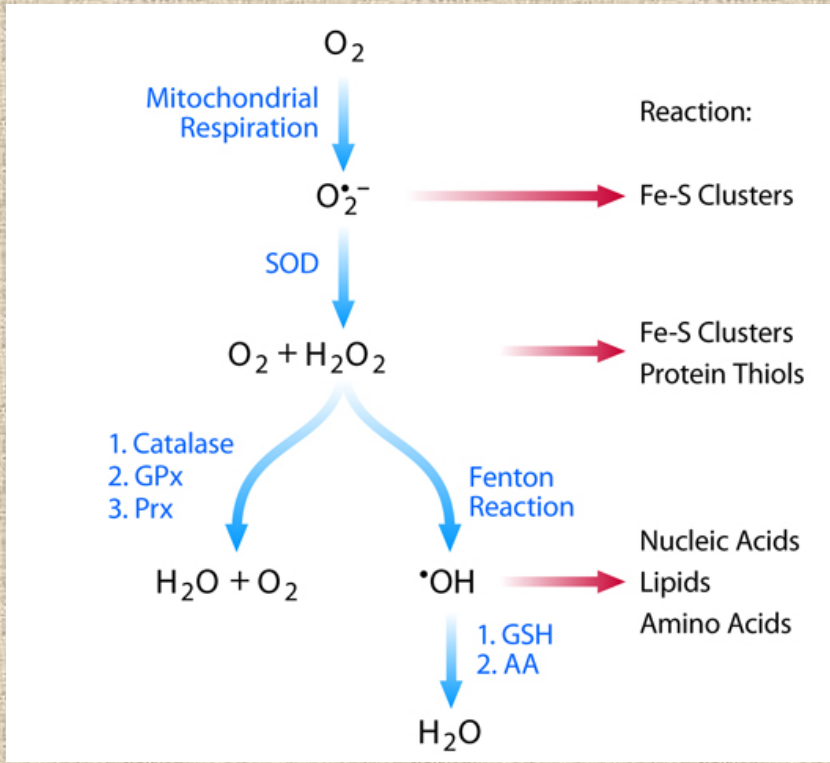
Endogenous ROS stress

- The O2•- also turns into H2O2 by Superoxide dismutase (SOD)

$$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$$

- The released Fe2+ can then reduce H2O2 to intracellular HO• (much higher reactive) (Fenton reaction)

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + HO^-$$



ROS stress from immune system

- On phagocytosis of Mtb, lung macrophages and neutrophils produce large quantities of ROS and RNS.
- NADPH oxidase in host cell catalyses the O2 using NADPH as electron donor, generating O2•–, as depicted in the following

$$2O_2 + NADPH \rightarrow O_2^{\bullet -} + NADP^+ + H^+$$

- Besides, hypochlorite ion (ClO–) could be generated by myeloperoxidase; ClO– is an extremely reactive oxidant and can lead to oxidative damage of lipids, proteins and DNA

$$Cl^- + H_2O_2 \rightarrow ClO^- + H_2O$$

RNS stress from immune system

- In response to mycobacterial infection, another major antimicrobial pathway that acts through inducible NO synthase is activated

$$\text{L} - \text{arginine} + \text{NADPH} + \text{H}^+ + \text{O}_2 \rightarrow \text{L-citrulline} + \text{NADP}^+ + \text{H}_2\text{O} + \text{NO}^\bullet \quad (10)$$

- Than NO• react with O2•– to produce highly reactive OONO−, than leads to the generation of NO−,•NO2, NO2−, N2O3, N2O4. wihch are all effective in killing Mtb

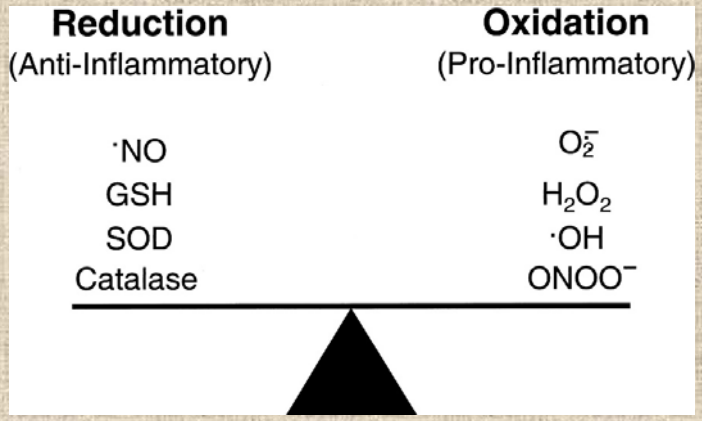
Hypoxia Stress in granuloma

- **Before granuloma formed**
  - Immune response (mononuclear cells and T lymphocytes)
  - Low pH
  - Oxidative stress
- **After granuloma matured (solid granuloma)**
  - **Hypoxia**
  - Low nutrition (foamy macrophage contains rich fatty acid in granuloma center)

Part III. Redox Homeostasis in *Mtb*

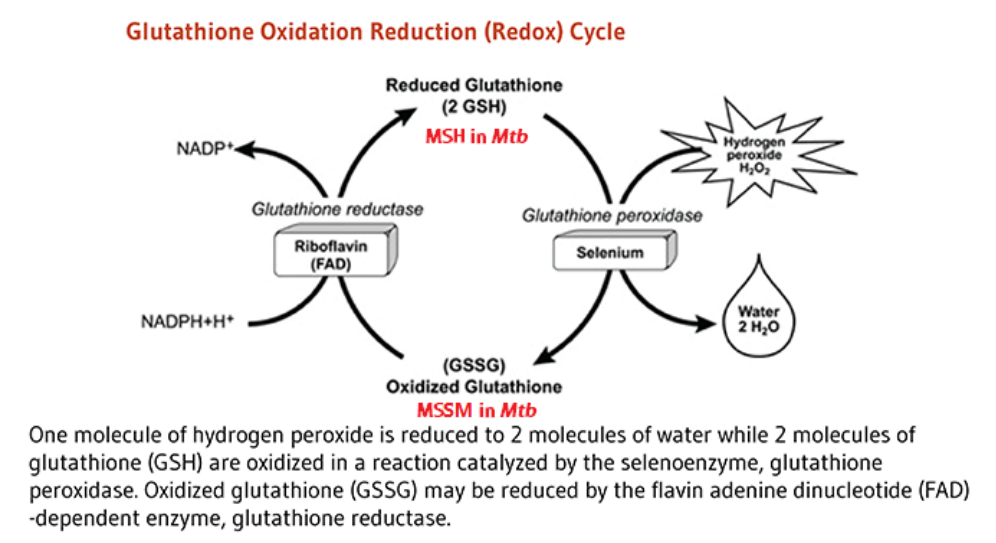
Redox Homeostasis in *Mtb*

- similar to other bacterial species, Mtb has evolved pathways to monitor redox signals (such as O2, NO and CO) and the alterations in all mentioned intra- and extracellular redox stresses.
- There are two basic types of strategy to keep redox homeostasis in *Mtb: non*-enzymatic and enzymatic



Non-enzymatic: THIOLS as Redox Buffers

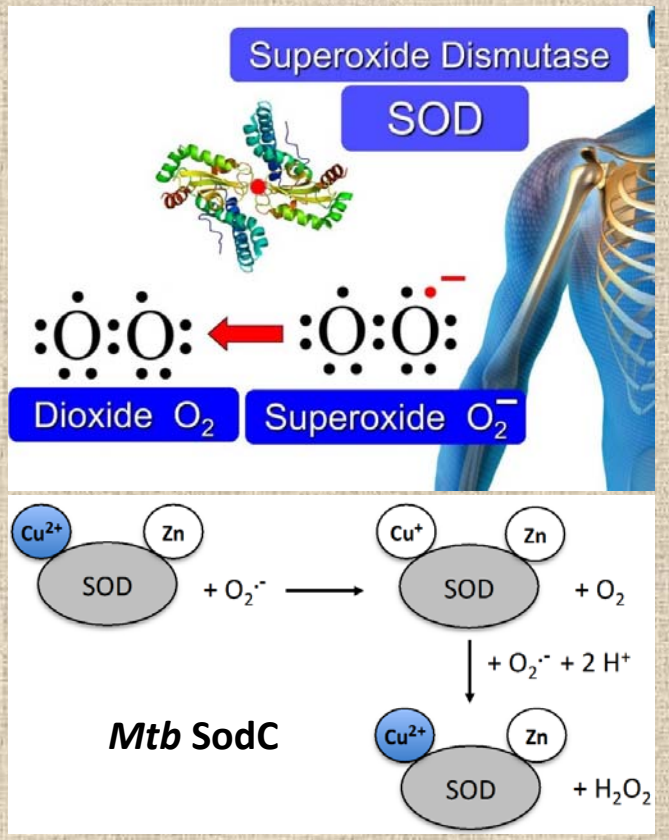
- Redox couples are present in all cells to keep the cytoplasm in a reduced, such as such as NAD+/NADH, NADP+/NADPH, FAD/FADH2
- The conventional redox couple glutathione (GSSG/2GSH) is absent in mycobacteria.
- Mycobacteria contain redox couples such as thioredoxin [TrxSS/Trx(SH)2], NADH/NAD+ and NADPH/NADP+, Rather, mycobacteria contain oxidised–reduced mycothiol (MSSM/ 2MSH) as the major redox buffer.



One molecule of hydrogen peroxide is reduced to 2 molecules of water while 2 molecules of glutathione (GSH) are oxidized in a reaction catalyzed by the selenoenzyme, glutathione peroxidase. Oxidized glutathione (GSSG) may be reduced by the flavin adenine dinucleotide (FAD) -dependent enzyme, glutathione reductase.

Enzymatic: Superoxide dismutases

- SODs produced by merely all cells to detoxify superoxide radicals. They catalyse the dismutation of O2•− into H2O2 and molecular oxygen.
- Mtb contains two SODs, an ironcontaining SOD called SodA and a Cu- and Zn-containing SOD called SodC.
- Its expression is enhanced by H2O2 exposure and on nutrient starvation, former study successfully showed that SodC protects Mtb against superoxide in vitro.



Enzymatic: Catalase peroxidase

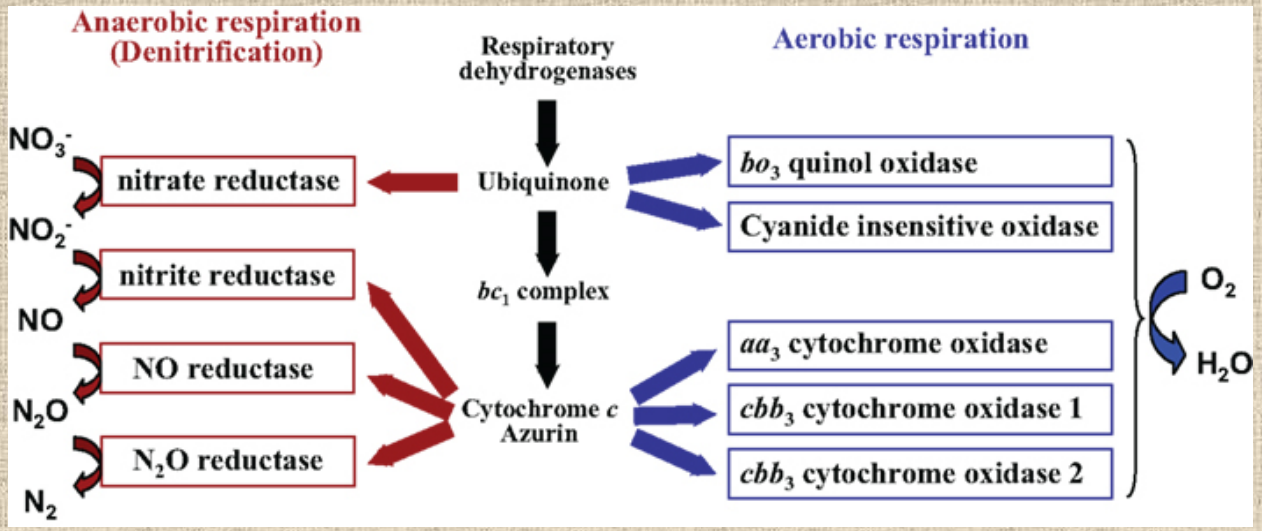
- Catalase peroxidases (Kat) are enzyme systems used to detoxify H2O2 into H2O and O2.
- Mtb owns one catalase, KatG that shows catalase, peroxidase and peroxinitritase activity.
- KatG has been demonstrated to be a virulence factor (Ref. 110) that mediates resistance against the prodrug INH.

Enzymatic: Methionine sulfoxide Reductases

- MSRs use NADPH, Trx and TrxR as the system to reduce methionine sulfoxide to methion
- Mtb contain two MSRs, one active on both free and peptidyl methionine-(S)-sulfoxide, and one or more MSRs active on peptidyl, but not free, methionine-(R)-sulfoxide ine and to protect bacteria against ROS and RNS

Change of Respiratory chain

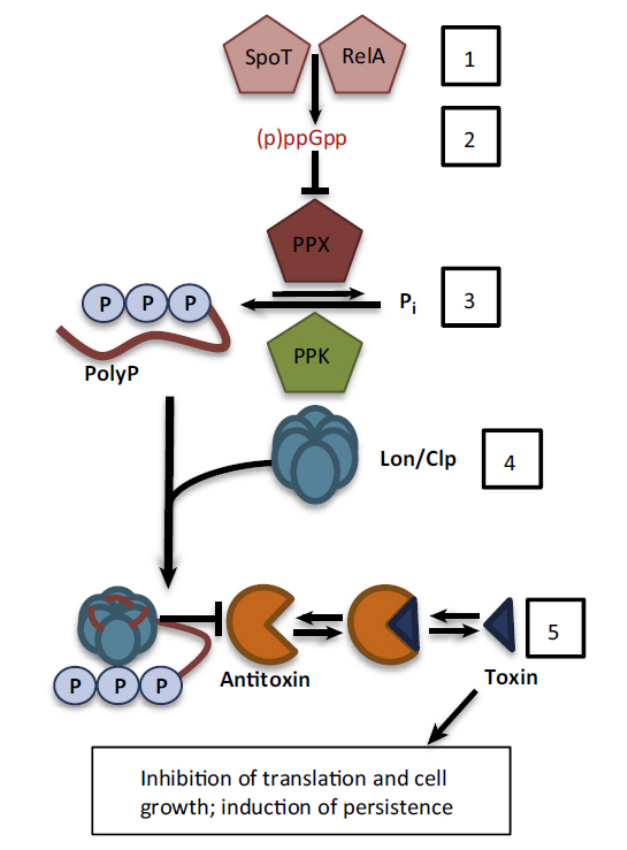
- Lack of terminal electron acceptors (O2)
- **Nitrate becomes new main electron acceptors**



- The respiratory chain is also changed, different from **Quinol & cytochrome** transferring the electron in aerobic situation, a series of **nitrogen reductase** form the new anaerobic electron transfer chain
- Nitrate is reduced by a nitrate reductase (*narGHJ*) and is then excreted by a nitrite extrusion protein (*narK1, narK2, narK3*)
- **Alternate** electron carriers in the hypoxic: **fumarate reductase**; probable **NAD(P)H dehydrogenases**; **ferredoxin** (These three parts were upregulated in transcription analysis)

Part IV. Redox sensing in *Mtb*

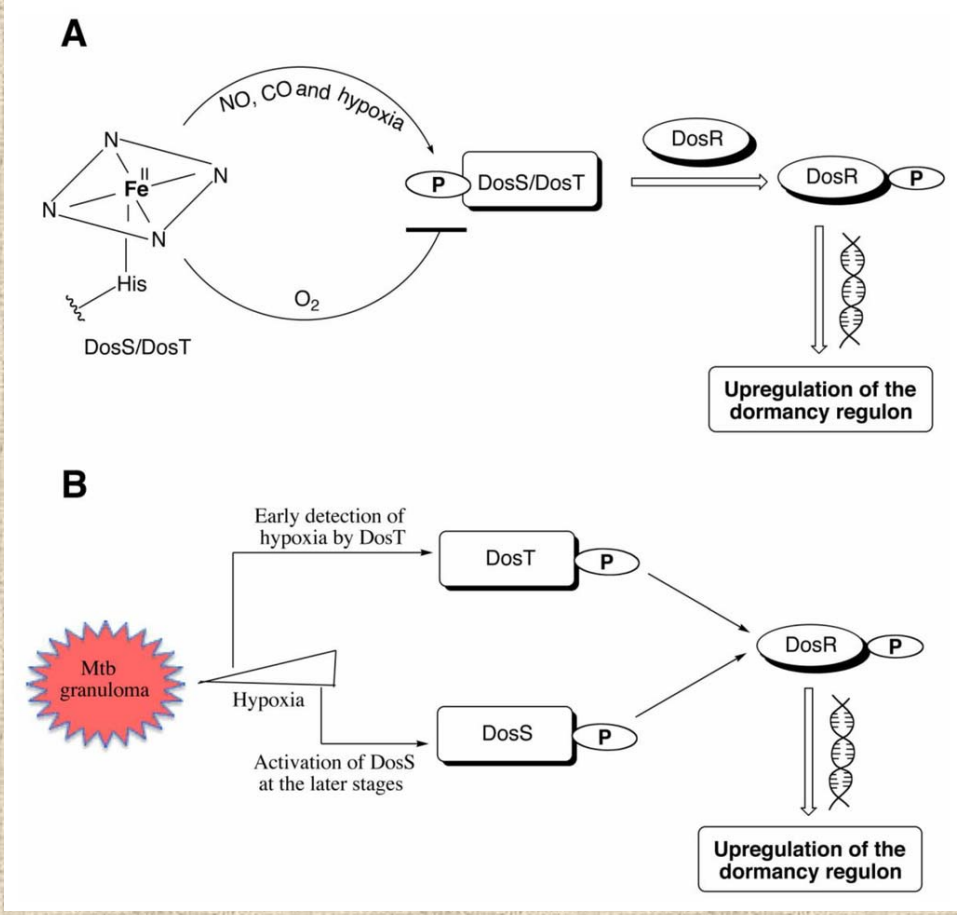
Stringent response : response to hypoxia

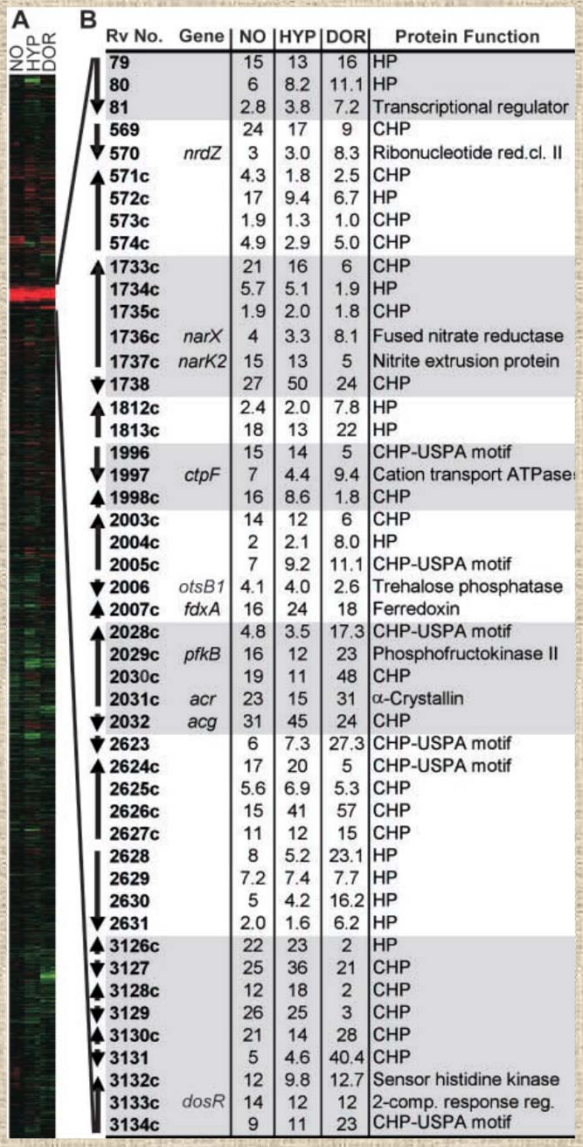


- In *Mtb,* the **ratio** of **amino-acylated tRNA** to **free tRNA** was the first regulatory response to amino acid & carbon starvation by **RelA**
- **ppGpp** is maintained in the cytosol by **RelA**
- ppGpp inhibits **polyphosphatase**, result in the accumulating of **PolyP**. **PolyP** interacts with **TA module**, finally **globally** affect **RNA polymerase,** then down-regulate gene expression

DosT/DosS/DosR three component sensor & regulon : response Oxidative stress

- DosT is a **gas sensor**, activated by **absence of oxygen** or the binding of **nitric oxide** and **carbon monoxide**. DosS is a **redox state sensor**
- Both DosT/DosS are **Kinase** to **Phosphorylate** DosR, resulting in downstream signaling
- Expression of DosR was induced by **DosT/DosS** two component sensor
- DosT & DosR activated in different time in hypoxia of granuloma





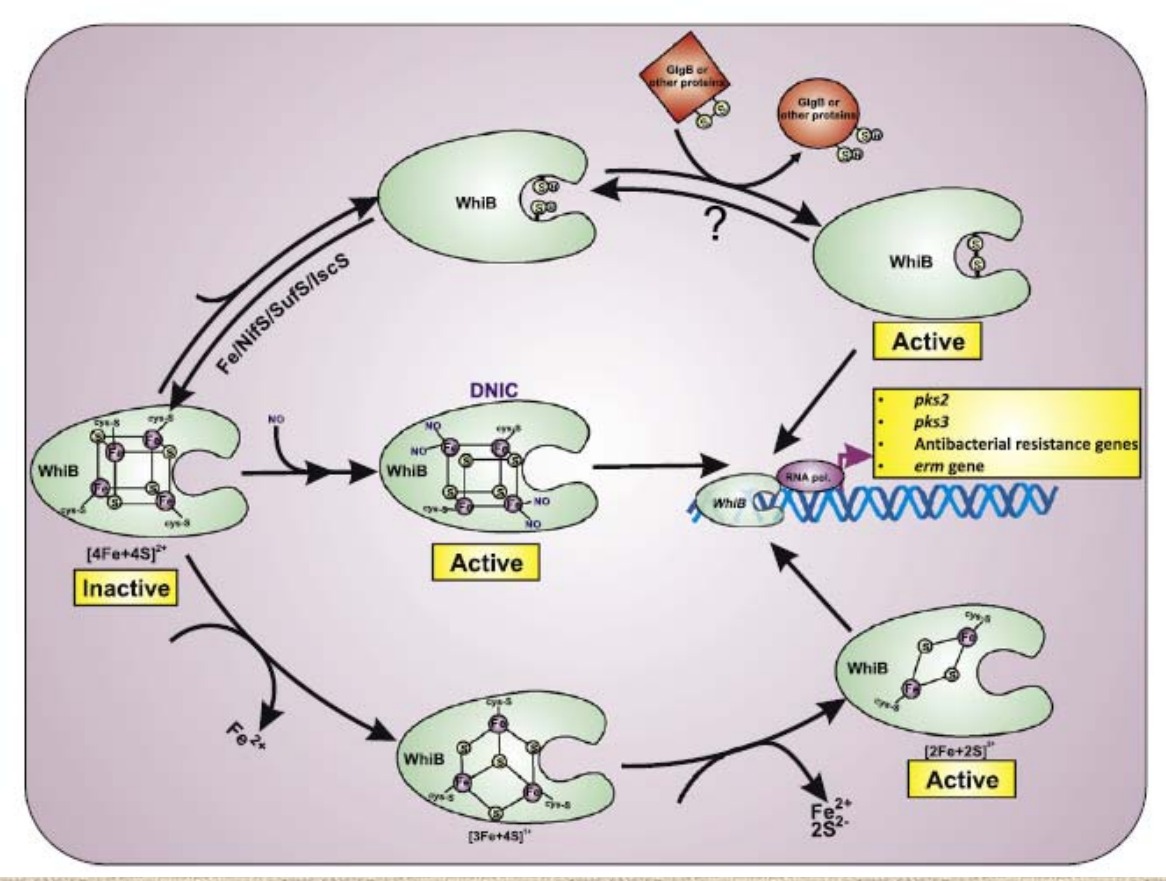
Rv No.	Gene	NO	HYP	DOR	Protein Function
79		15	13	16	HP
80		6	8.2	11.1	HP
81		2.8	3.8	7.2	Transcriptional regulator
569		24	17	9	CHP
570	*nrdZ*	3	3.0	8.3	Ribonucleotide red.cl. II
571c		4.3	1.8	2.5	CHP
572c		17	9.4	6.7	HP
573c		1.9	1.3	1.0	CHP
574c		4.9	2.9	5.0	CHP
1733c		21	16	6	CHP
1734c		5.7	5.1	1.9	HP
1735c		1.9	2.0	1.8	CHP
1736c	*narX*	4	3.3	8.1	Fused nitrate reductase
1737c	*narK2*	15	13	5	Nitrite extrusion protein
1738		27	50	24	CHP
1812c		2.4	2.0	7.8	HP
1813c		18	13	22	HP
1996		15	14	5	CHP-USPA motif
1997	*ctpF*	7	4.4	9.4	Cation transport ATPase
1998c		16	8.6	1.8	CHP
2003c		14	12	6	CHP
2004c		2	2.1	8.0	HP
2005c		7	9.2	11.1	CHP-USPA motif
2006	*otsB1*	4.1	4.0	2.6	Trehalose phosphatase
2007c	*fdxA*	16	24	18	Ferredoxin
2028c		4.8	3.5	17.3	CHP-USPA motif
2029c	*pfkB*	16	12	23	Phosphofructokinase II
2030c		19	11	48	CHP
2031c	*acr*	23	15	31	α-Crystallin
2032	*acg*	31	45	24	CHP
2623		6	7.3	27.3	CHP-USPA motif
2624c		17	20	5	CHP-USPA motif
2625c		5.6	6.9	5.3	CHP
2626c		15	41	57	CHP
2627c		11	12	15	CHP
2628		8	5.2	23.1	HP
2629		7.2	7.4	7.7	HP
2630		5	4.2	16.2	HP
2631		2.0	1.6	6.2	HP
3126c		22	23	2	HP
3127		25	36	21	CHP
3128c		12	18	2	CHP
3129		26	25	3	CHP
3130c		21	14	28	CHP
3131		5	4.6	40.4	CHP
3132c		12	9.8	12.7	Sensor histidine kinase
3133c	*dosR*	14	12	12	2-comp. response reg.
3134c		9	11	23	CHP-USPA motif

DosR regulon

- Now 53 genes was found regulated by *dosR*. Including **4 transporters**, **2 Nitrate respiratory chain**, **2 regulator**
- Nearly 60% of the genes do not have an annotated function, by sequence & domain comparison, 11 involved in **carbohydrate and fatty acid metabolism**; 8 in **electron transfer**

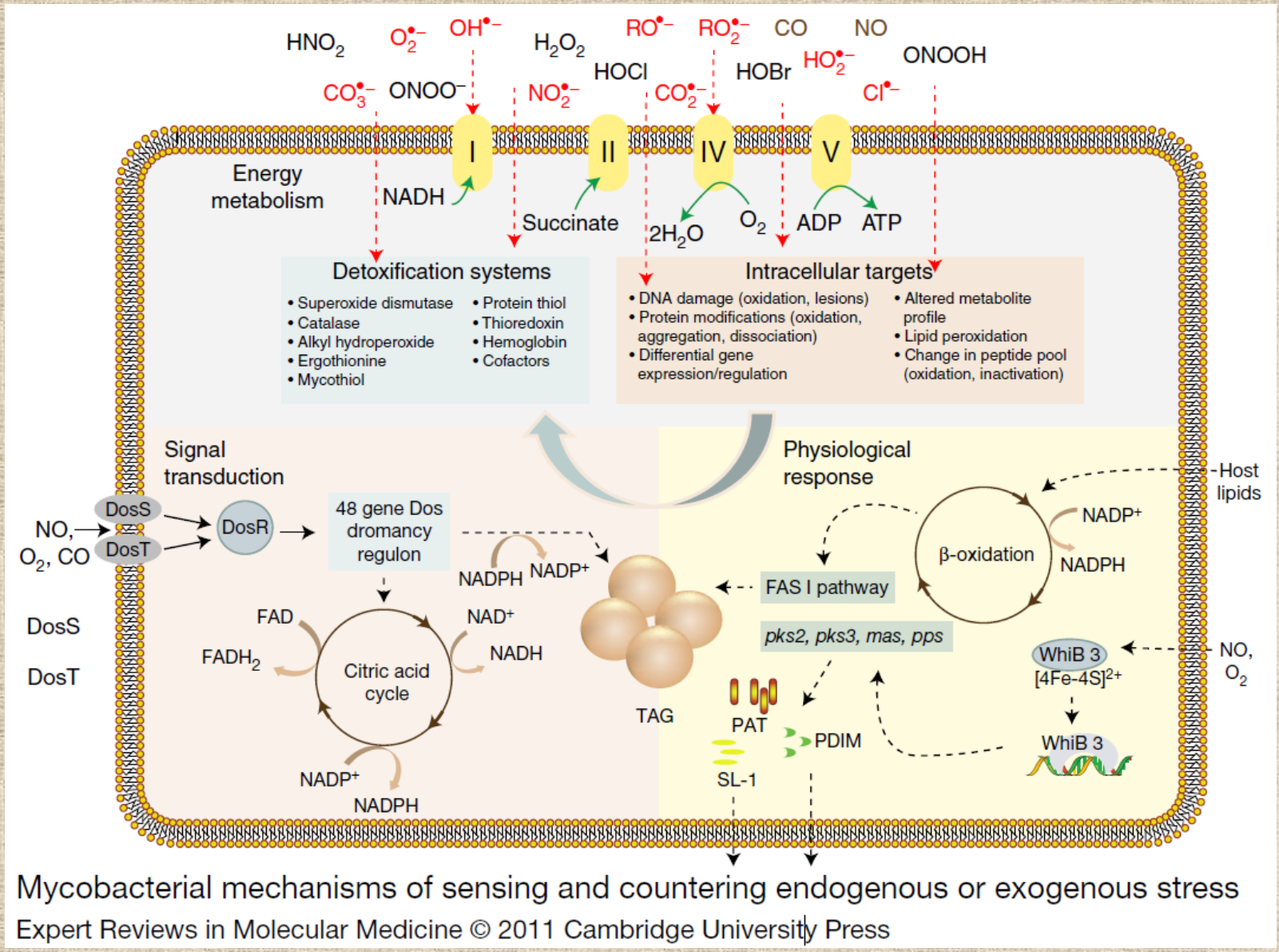
WhiB proteins as iron–sulfur cluster-based sensors

- WhiB3 is an oxygen and NO sensor
- WhiB binds a[4Fe–4S]2+ cluster, which exposure to oxygen or NO leads to activate to a [2Fe– 2S]2+
- This changes in WhiB proteins that enhance the DNA-binding activity of WhiB3



Summary:

Mycobacterial mechanisms of sensing and countering Oxidative stress



Mycobacterial mechanisms of sensing and countering endogenous or exogenous stress

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Thank you!