Metabolic characteristics in dormant *Mycobacterium tuberculosis*——an essential physiology shift during latent tuberculosis infections.

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Outline

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  II. ATP synthesis
  III. Respiratory chain
  IV. Redox balance
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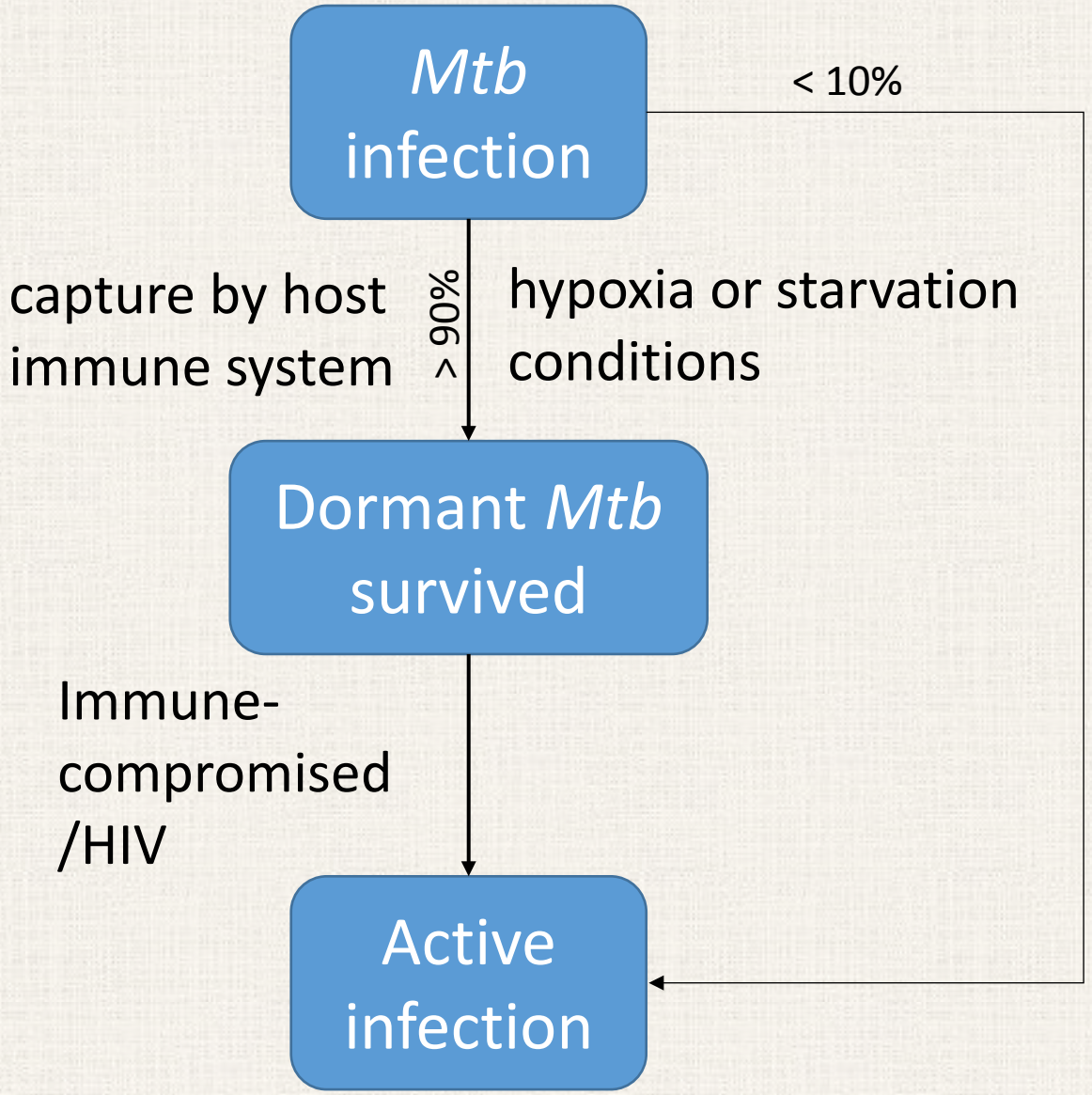
  I. stringent response
  II. DosR regulation system
- Mtb dormancy & molybdenum cofactor

Part I. Background of *Mtb* dormancy

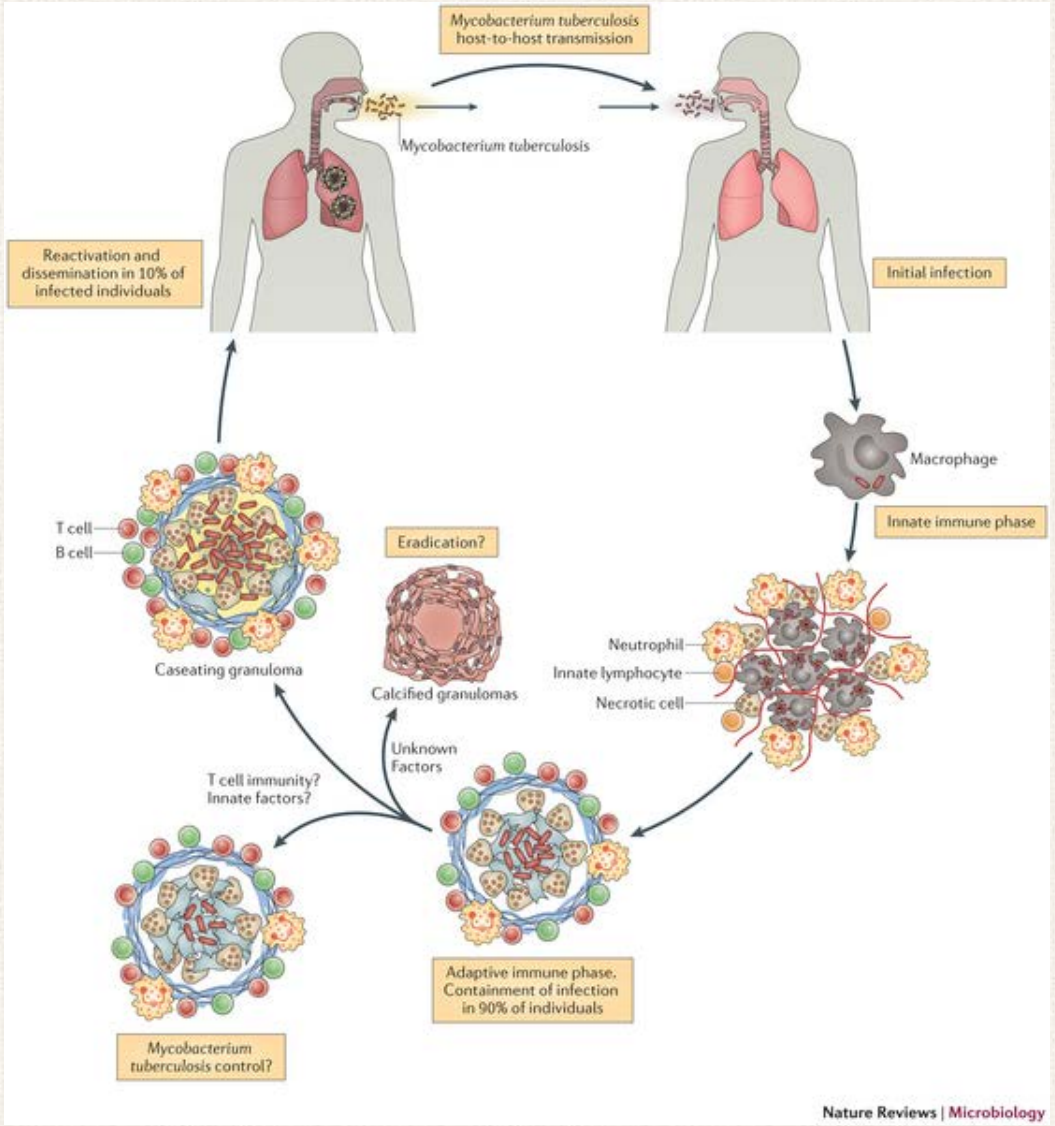
latent infections of tuberculosis & *Mtb dormancy*

- The incredible **success** of *Mtb* with **Widely spread** & **long-term treatment** largely due to the **latent infection of tuberculosis (LTBI)**
  - Many proof showed **dormant *Mtb*** is the main state of pathogen during **the latent/persistent infections**
  - Dormant *Mtb* is induced during **infection process** in host cell and might remain latent state for **decades**
- When in more **favorable conditions**→ resuscitate to active→active infection



Advantage of dormancy during *Mtb* infection

- **Evade most immune stress** from host cells **for decades**
- Difficult to cure: at least a **six mouths treatment** & **relapse** (remain few dormant cell in entire Mtb)
- **Higher** chance to accumulate **Drug-resistance mutants** (multi- or Extensively-drug resistant tb)



A typical latent infection of Mtb

"captured→multiplication → enter dormancy → living in macrophage → resuscitation → active infection"

Stress and living environment in host cells

- **Before granuloma formed**
  - Immune response (mononuclear cells and T lymphocytes)
  - Low pH
  - Oxidative stress (NO/CO produced by macrophage)

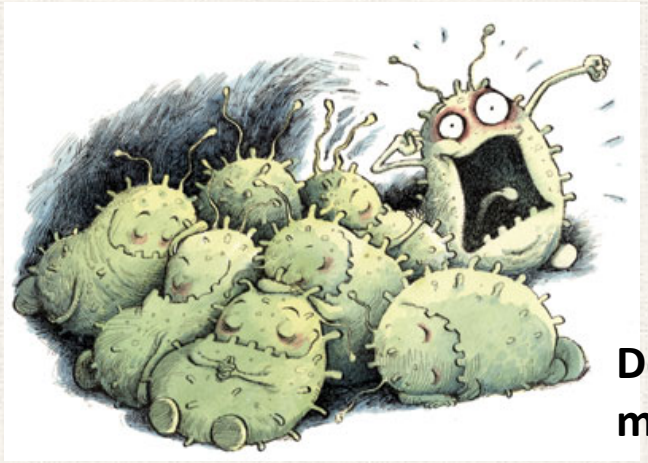
- **After granuloma matured (solid granuloma)**
  - **Hypoxia**
  - Low nutrition (foamy macrophage contains rich fatty acid in granuloma center)

Basic **features** of dormant *Mtb*

- **Low metabolism level** & **Non-replicative state**
  - **Thickening** of the cell wall
  - **Shutdown** of most **transcription** & **protein synthesis**
  - Decrease of **ATP synthesis**

Hibernation of mammal

- **Maintaining respiration** & **the integrity of cell membrane**
  - Switch **electron transfer/ accepter**
  - Alter **carbon metabolism** pathway
  - Maintain the **proton gradient** across the membrane

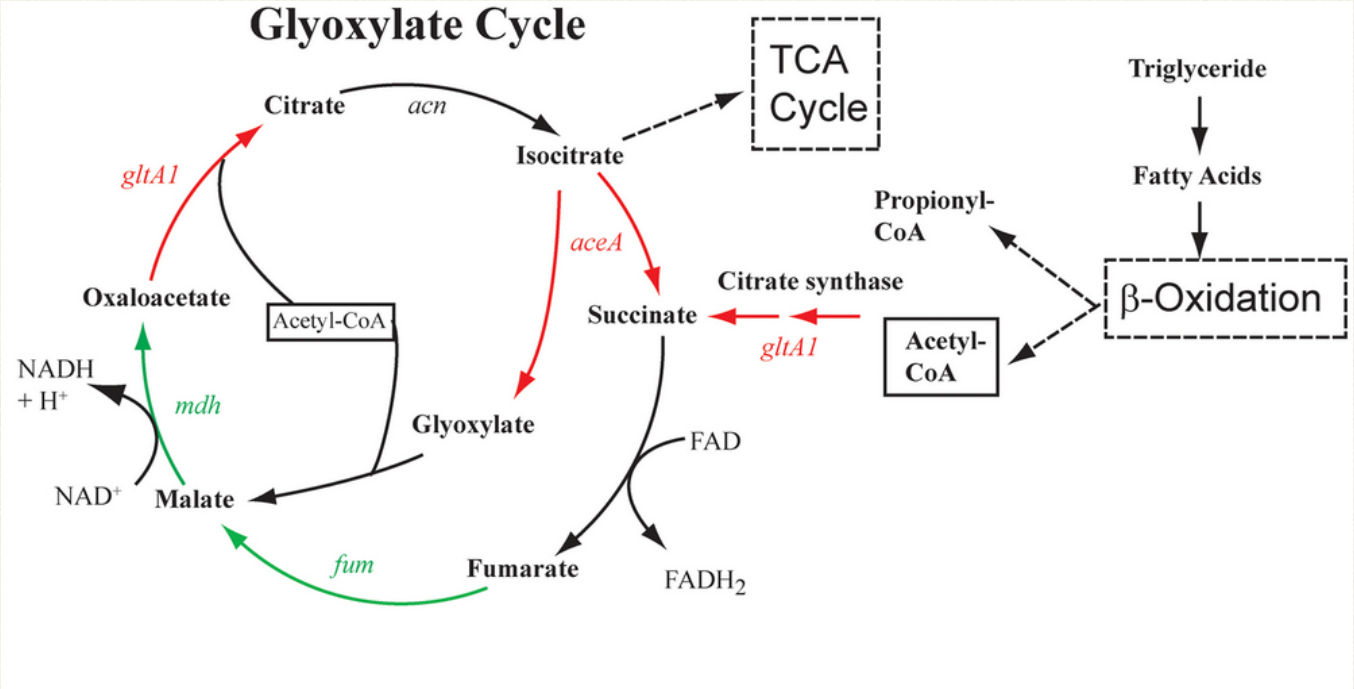


Dormancy of microbes

Part II. Metabolic changes in dormant *Mtb*

Carbon metabolism

- The dual stresses in macrophage: hypoxia/unbalanced nutrient pool rich in fatty acids and poor in carbohydrates
- **Five-step beta-oxidation pathway** & **Glyoxylate cycle** was **up regulated** but **carbohydrates metabolism** was **turned down**
- Isocitrate lyase (ICL) is reported plays another role in suppressing cell apoptosis of macrophage



Microarray analysis demonstrated changes in expression of genes involved in glyoxylate cycle. Daniel J, et al. (2009)

ATP synthesis

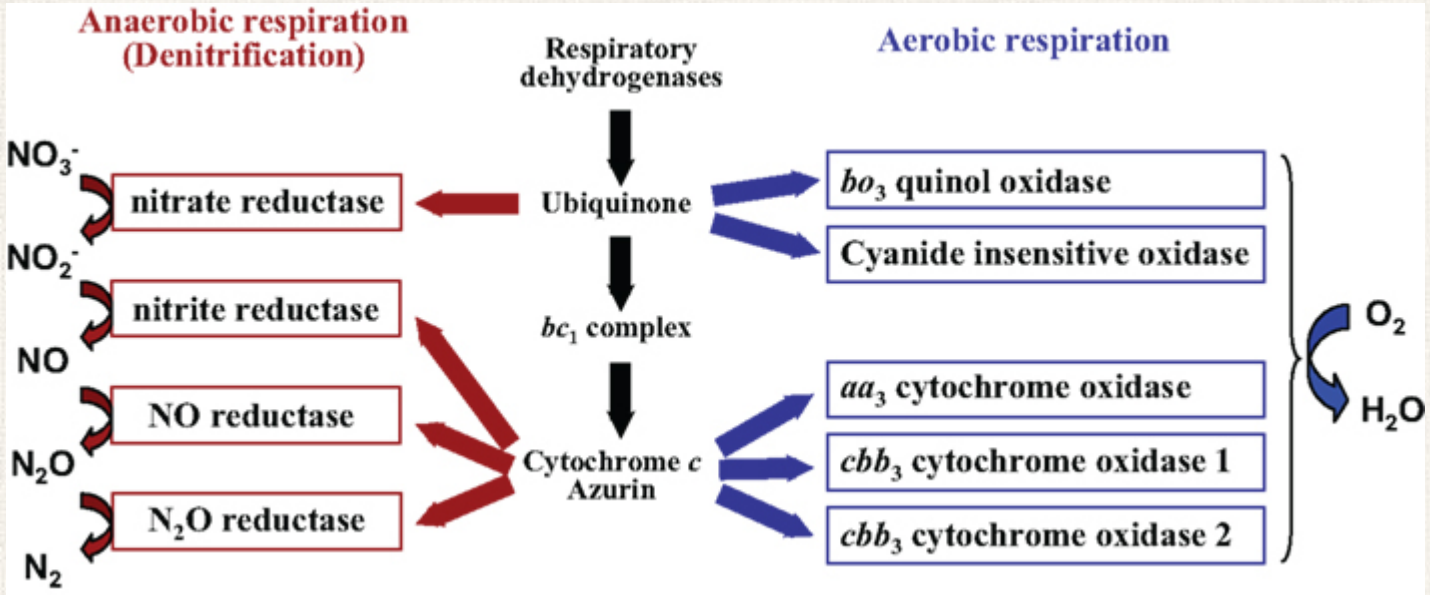
- Cell-wide downregulation of metabolism & nonreplicating
- In the **transcription analysis**, the most significant **ATP synthase cluster atpA-H** strongly down regulated, suggesting a **global down-regulation** of the ATP synthesis
- ATP is a **scarce resource** in the dormant cell

Rv number	Up-regulation Score	Down-regulation Score	Growth-attenuation Score	Gene ID
F1FO ATP synthase				
Rv1308	0	-14.68	4.95	atpA
Rv1304	0	0.97	-15.56	4.825 atpB
Rv1311	0	3.465	-9.49	3.66 atpC
Rv1310	0	0	-15.32	4.55 atpD
Rv1305	0	0	-12.87	2.45 atpE
Rv1306	0	0	-15.845	4.745 atpF
Rv1309	0	0	-13.2	4.525 atpG
Rv1307	0	0	-11.865	4.59 atpH

Scoring and annotations for ATP synthase subunit genes. Searchable table of scoring results for genes encoding various ATP subunits in the Mycobacterium tuberculosis *genome. Dennis J Murphy (2007)*

Change of Respiratory chain

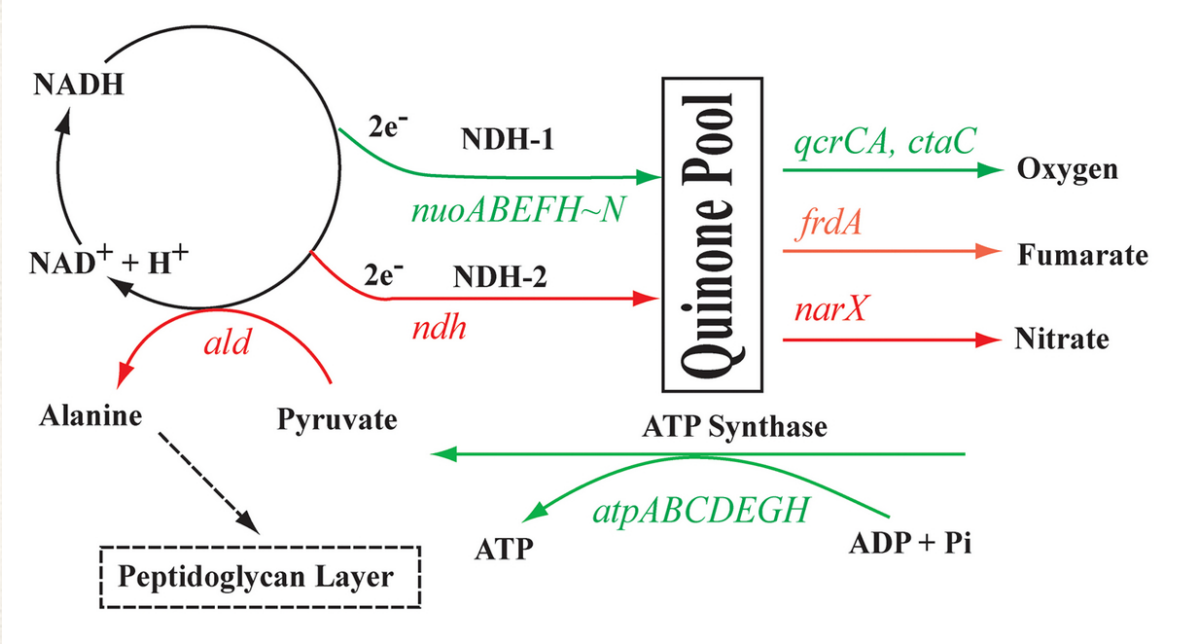
- Lack of terminal electron acceptors (O2)
- **Nitrate becomes new main electron acceptors**



- 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)

Redox balance

- Lack of terminal **electron acceptors**, NADH dehydrogenase I subunits and ubiquinol–cytochrome C complex were **repressed**
- Beta-oxidation of fatty acid, NADH was produced. To ensure the ratio of NADH/NAD+, the NADH dehydrogenase II is **upregulanted**. To help the proton transferring, expression of lower-efficiency anaerobic respiration enzymes (frdA, narG/X, nirA) are also **increased**
- Besides**, aminotransferase** is upregulanted, expend NADH and take part in other anabolism such as thickening of the cell wall



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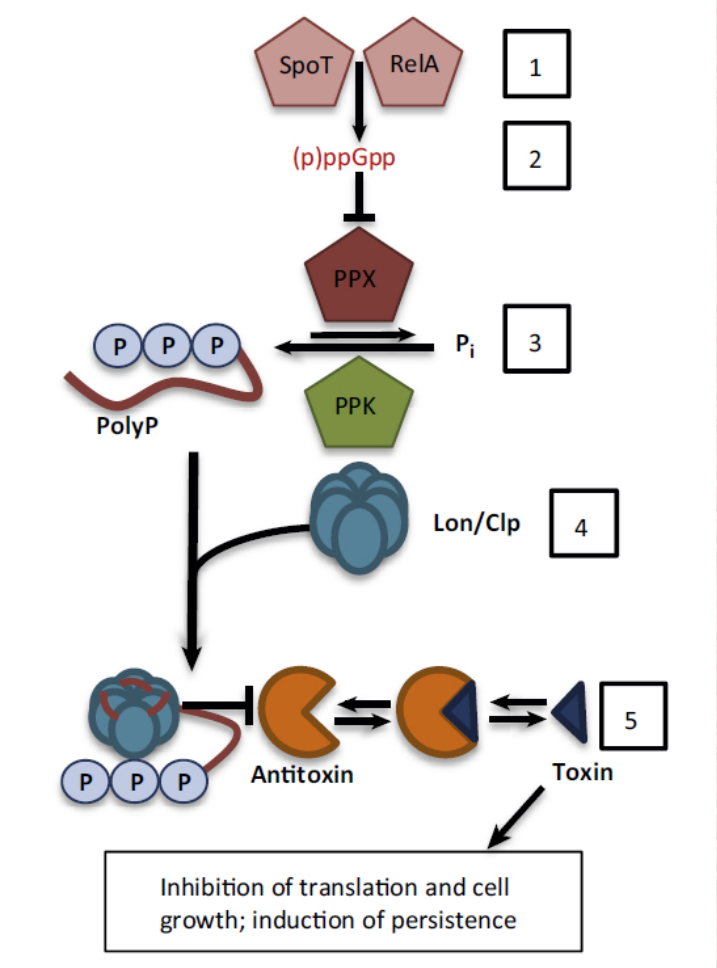
Part III. Stress sensor & regulation in Mtb dormancy

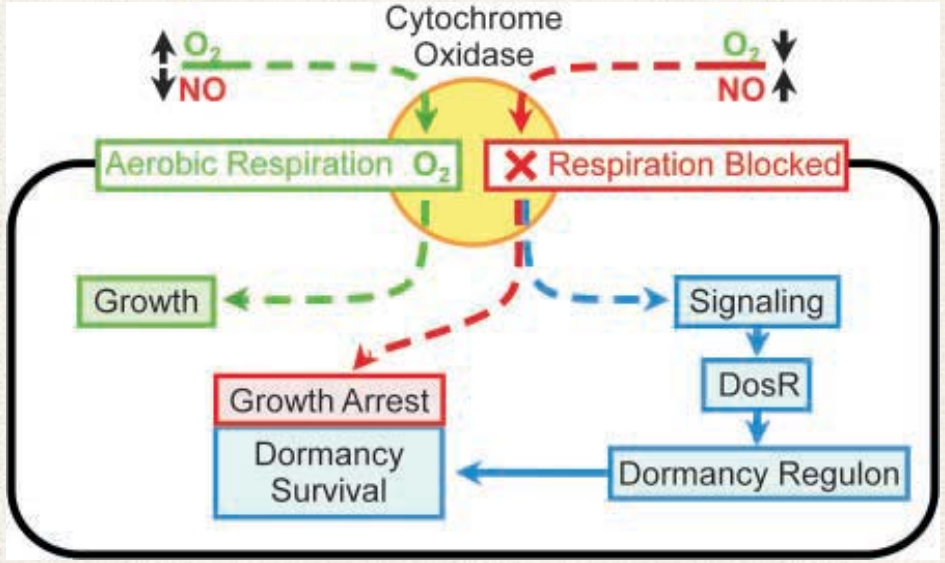
Stringent response : response to low nutrition



- 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

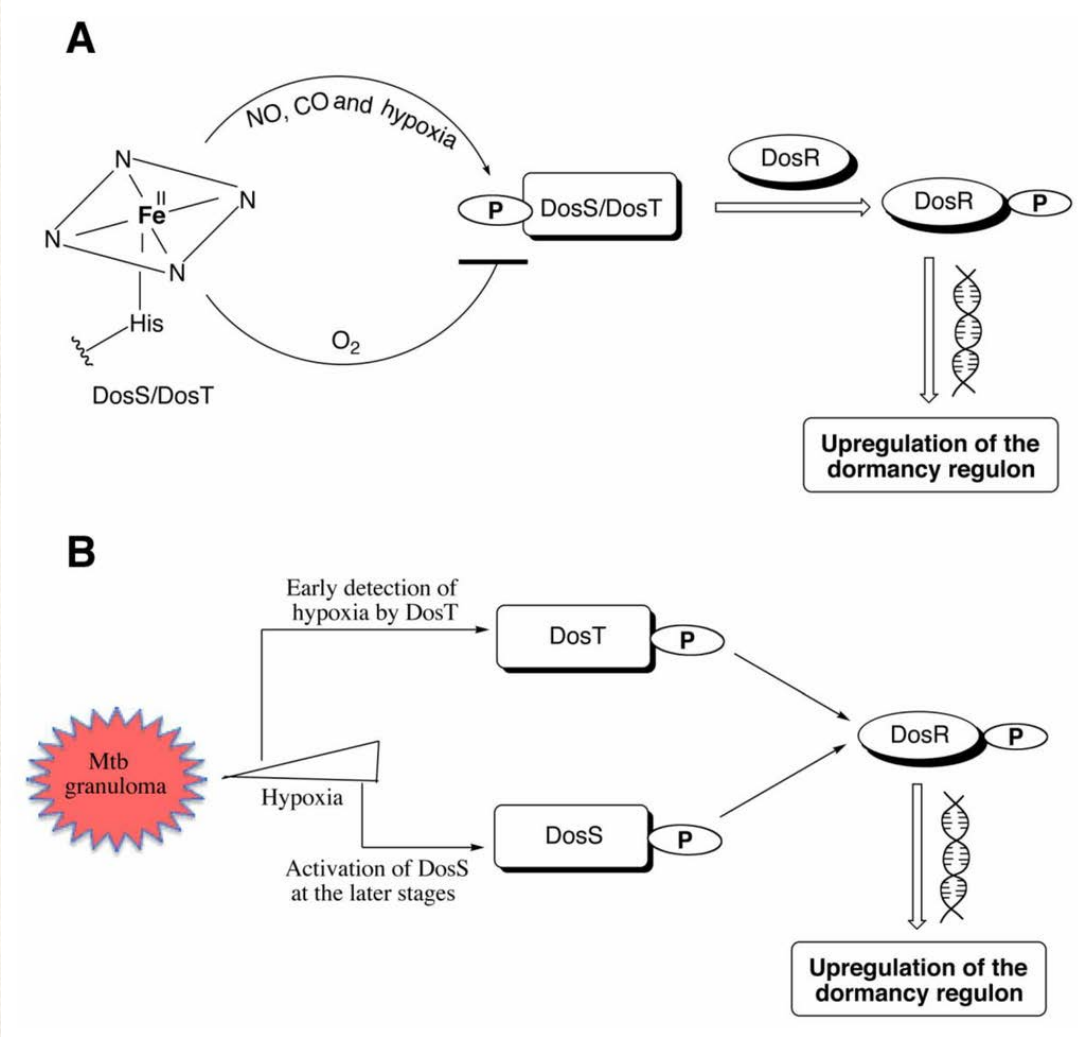
DosT/DosS two component sensor *&* DosR regulon : response to hypoxia & Oxidative stress

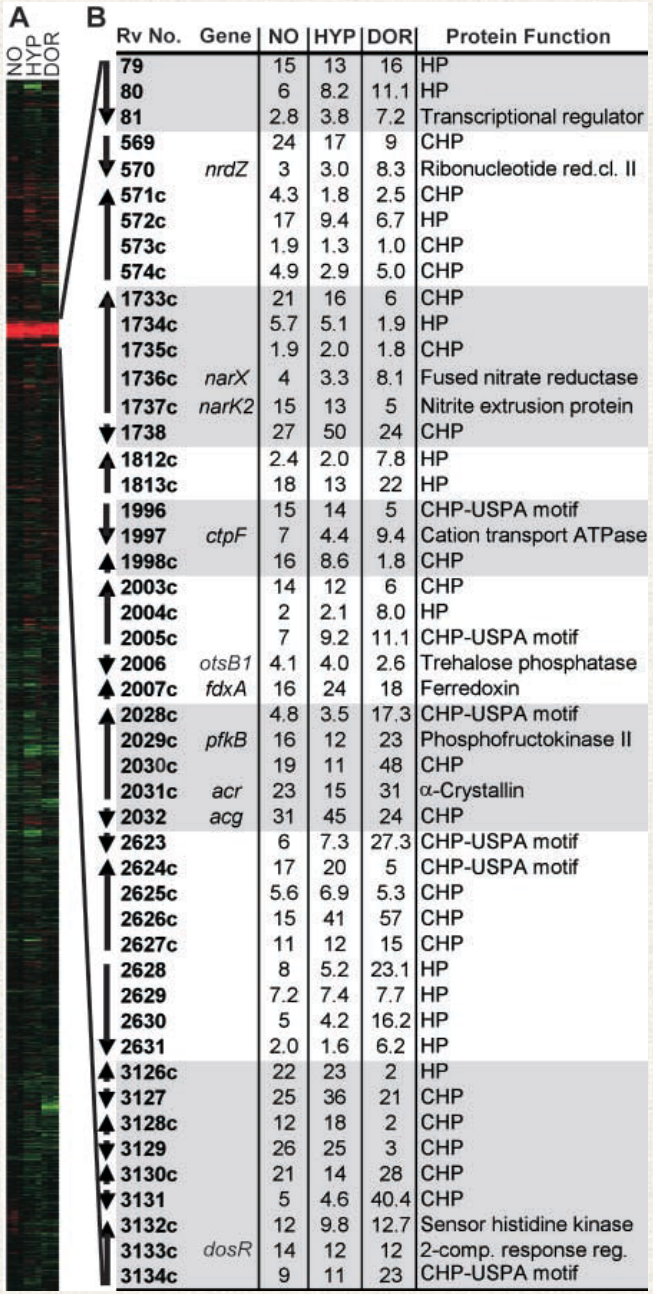
- DosR system seems to be the secondary important dormancy regulator in hypoxia, next to the directly growth arrest by lack of O2 (red color)
- Expression of DosR was induced by **DosT/DosS** two component sensor
- DosR controls a regulon of **more than 53 genes**, including enzyme in metabolism, respiration pathway, even two regulators



DosRST two-component system

- 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
- DosT & DosR activated in different time in hypoxia of granuloma





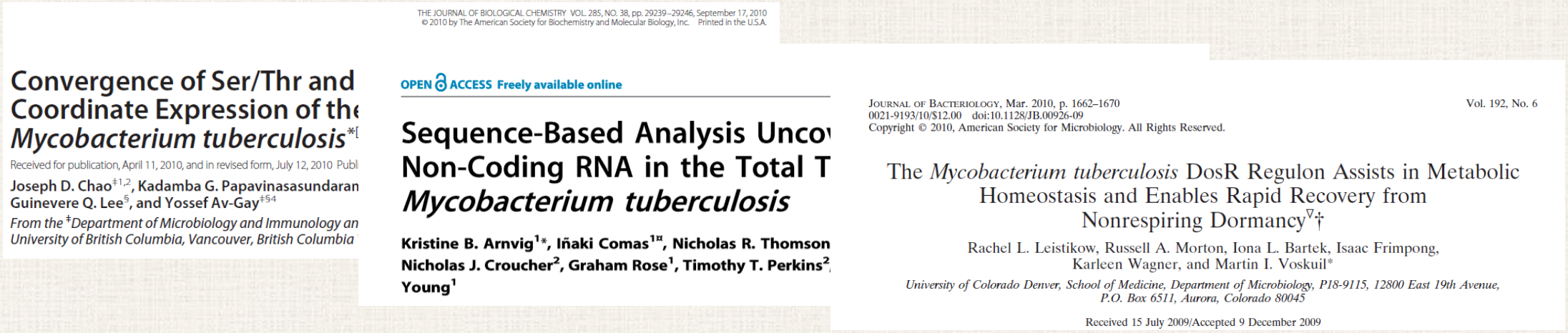
Rv No.	Gene	NO	HYP	DOR	Protein Function
79		15	13	16	HP
80		6	8.2	11.1	HP
81		2.8	3.8	7.2	Transcriptional regulator
569		24	17	9	CHP
570	*nrdZ*	3	3.0	8.3	Ribonucleotide red.cl. II
571c		4.3	1.8	2.5	CHP
572c		17	9.4	6.7	HP
573c		1.9	1.3	1.0	CHP
574c		4.9	2.9	5.0	CHP
1733c		21	16	6	CHP
1734c		5.7	5.1	1.9	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** (cascading signal)
- 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**

New insights of the DosR regulon

- In Zheng X' work *(2010)*, DosR showed an additional signaling networks involving **Serine/threonine protein kinases**
- In Thomson NR' work(2011), they discovered **noncoding small RNAs** that appear to be under DosR control.
- In Voskuil MI' work(2013), DosR is required for *M. tuberculosis* **exits the dormant state**



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Convergence of Ser/Thr and
Coordinate Expression of the
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Sequence-Based Analysis Uncov
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Kristine B. Arnvig, Iñaki Comas, Nicholas R. Thomson
Nicholas J. Croucher, Graham Rose, Timothy T. Perkins,
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The *Mycobacterium tuberculosis* DosR Regulon Assists in Metabolic Homeostasis and Enables Rapid Recovery from Nonrespiring Dormancy†

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Several Models of Mtb dormancy

- Wayne's anaerobic model
- rather reflects an adaptive response to low oxygen conditions than the state of true deep dormancy

- Other models
- based upon culturing M. tuberculosis in Sauton's medium without potassium.Under these conditions, more than 99% of bacterial cells transit to dormant, non-culturable state during a prolonged, 60-d stationary phase.
- gradual acidification of the medium, resulting in a massive accumulation of ovoid cells with the properties closely resembling those predicted for dormant bacteria.

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Thank you!