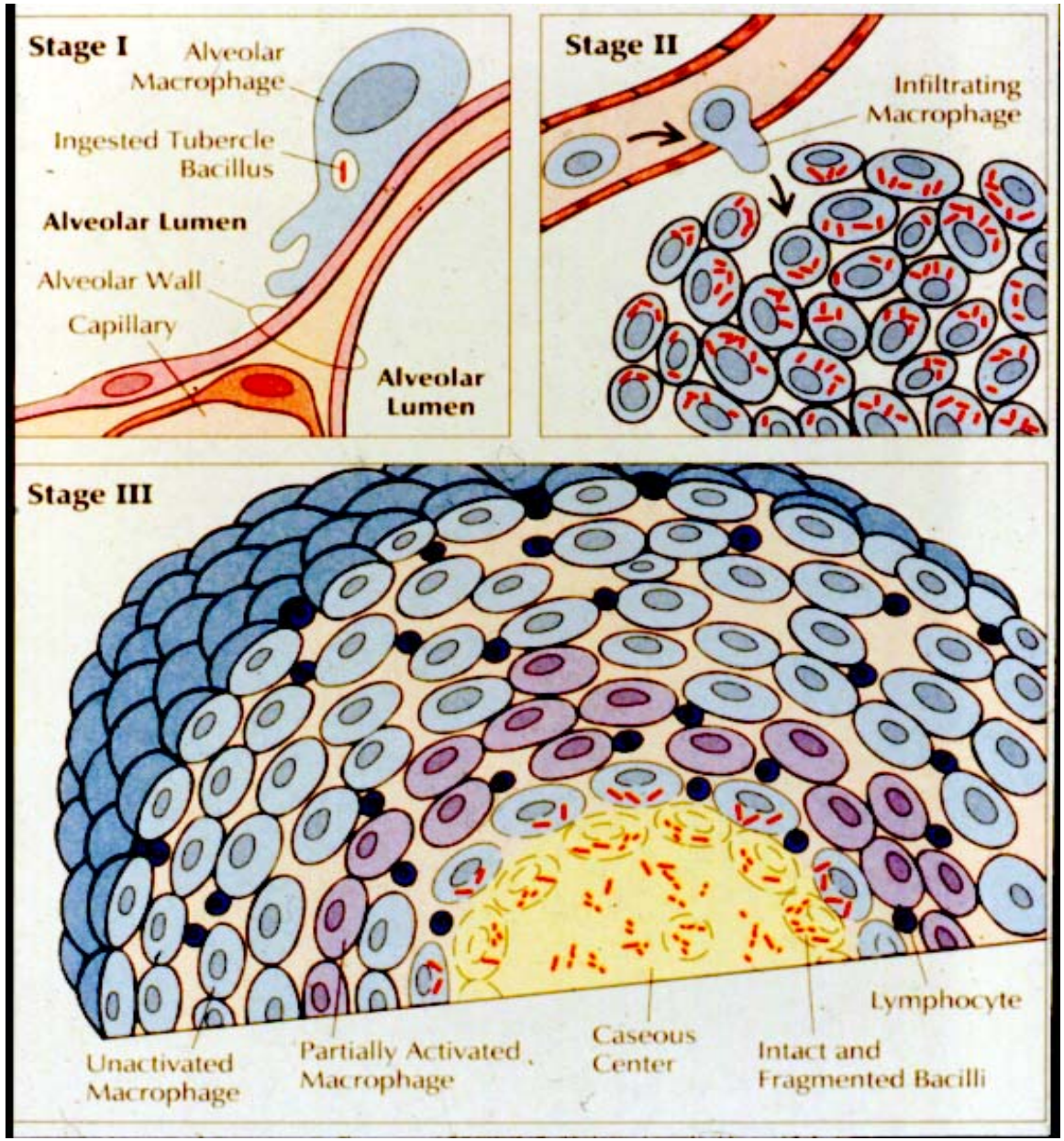
12th Annual Conference
IUATLD-NAR

- Dr. David Sherman has no financial relationship with companies who have provided support to this meeting that suggests a personal conflict of interest in relations to the planning for the above captioned CME Event.

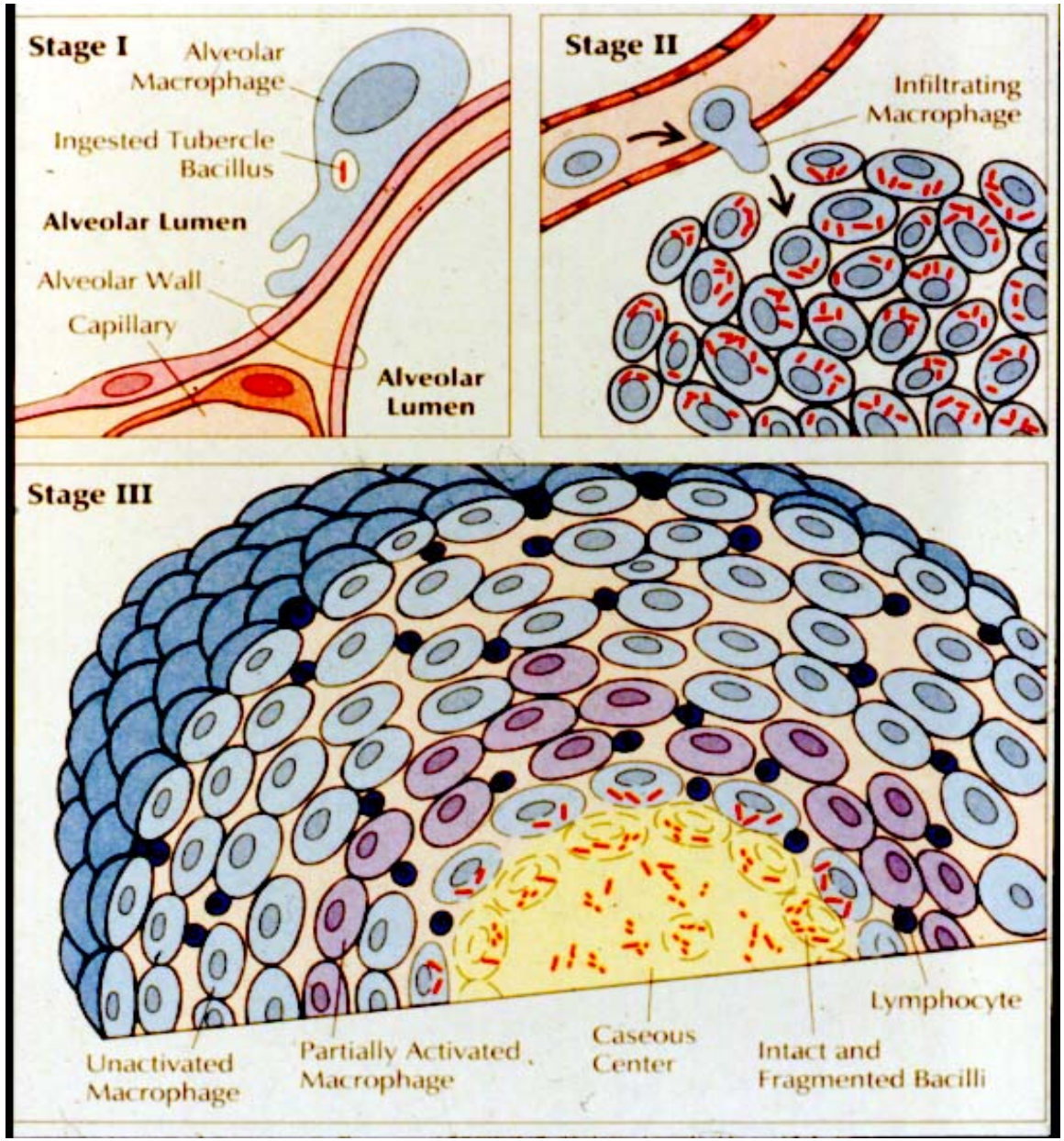
Sleeping and waking: Deciphering the game plan of chronic *M. tuberculosis*

David R. Sherman
Director, TB Program

Stages of TB pathogenesis



Dannenberg, Hosp Pract 1993;28:51-8.



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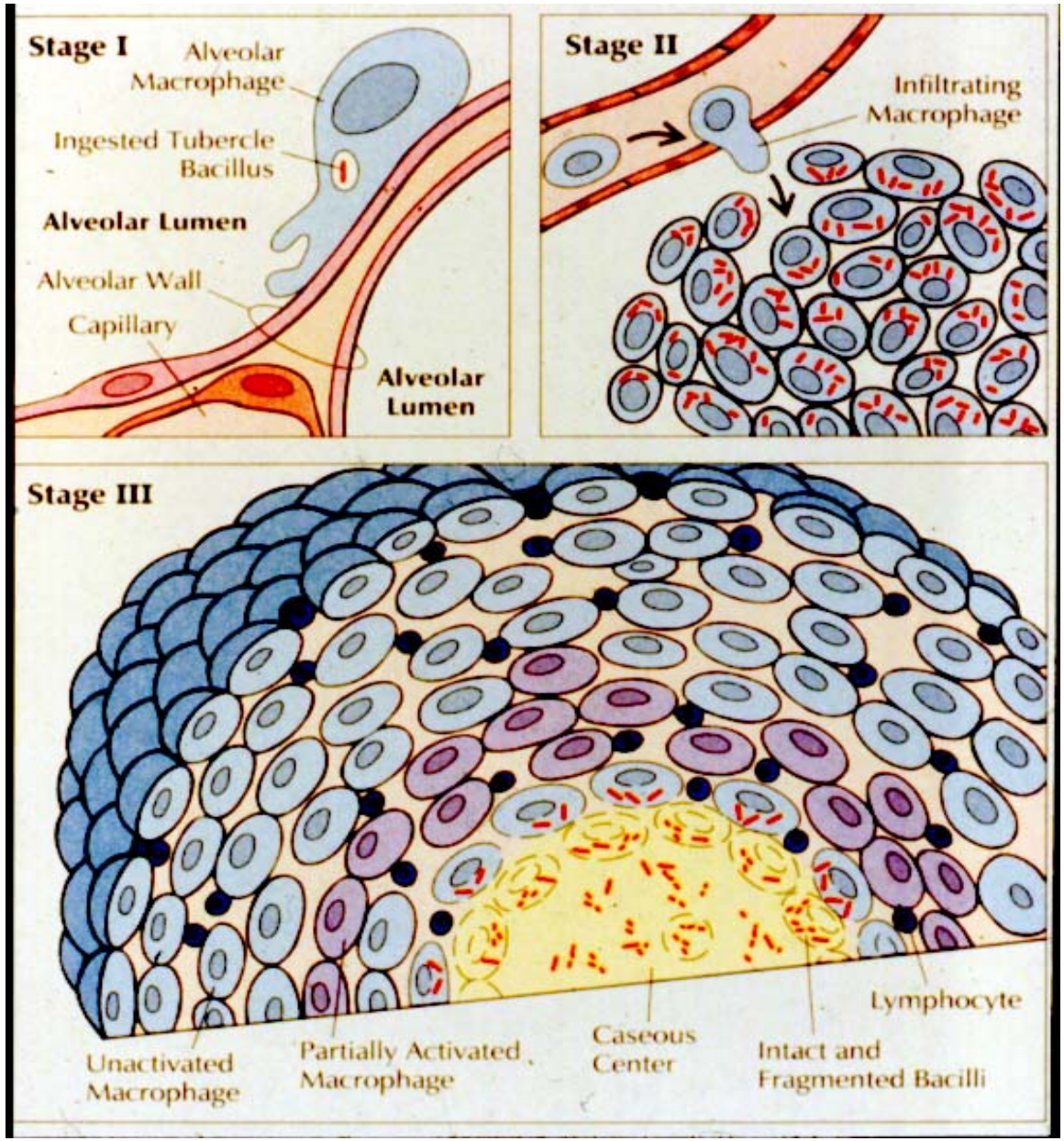
TB granuloma "facts"

- MTB stops dividing; isn't eradicated.
- MTB growth limited by hypoxia.
- Granuloma is the primary unit of host defense.



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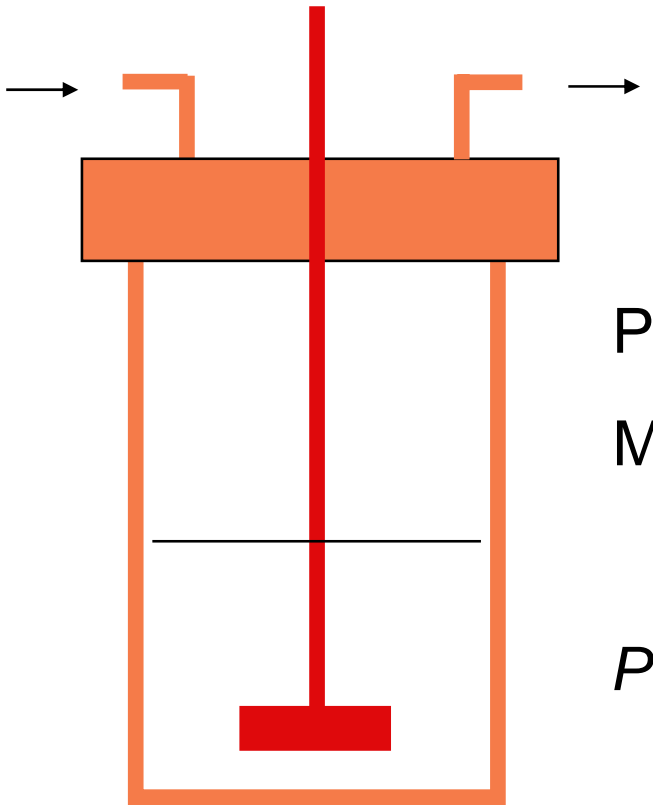
MTB defined hypoxic culture



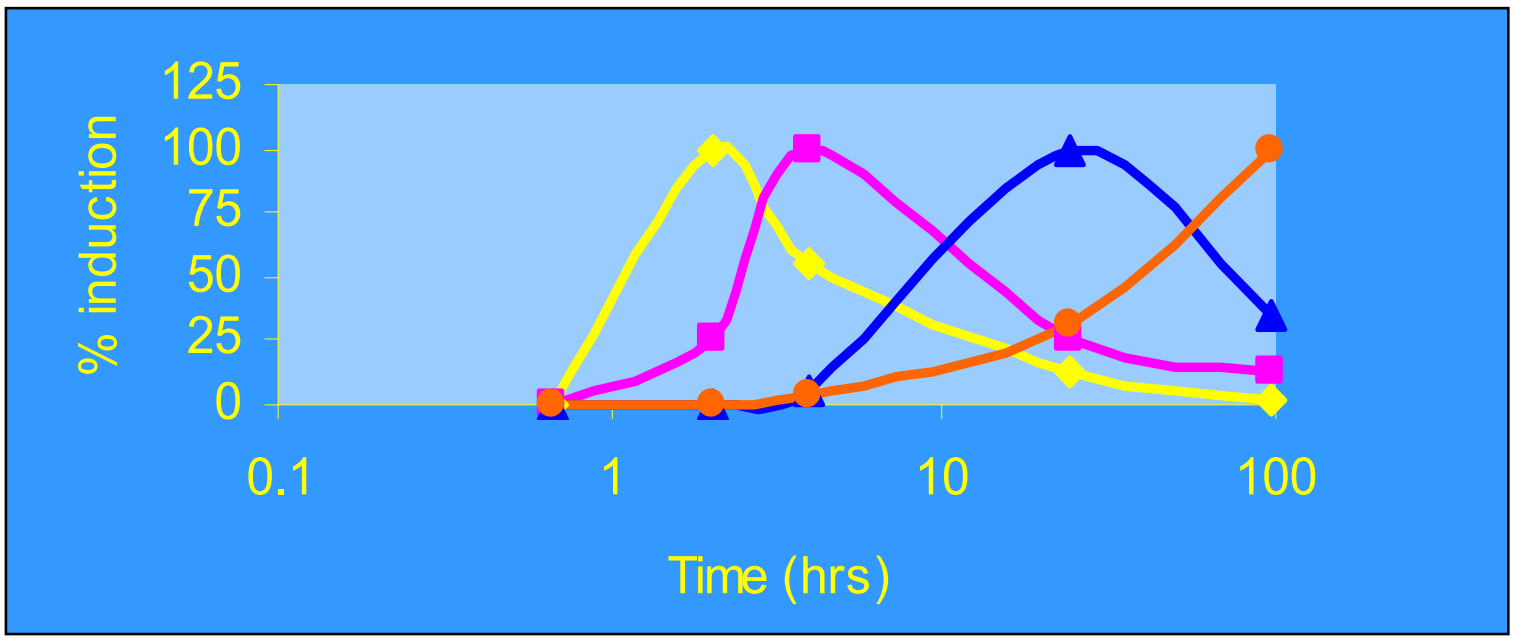
Pre-mixed gas is continuously introduced.

MTB maintained in a non-replicative state.

Purify RNA for analysis by microarray.

MTB hypoxic gene expression: first assumptions

Collect RNA, analyze by microarray

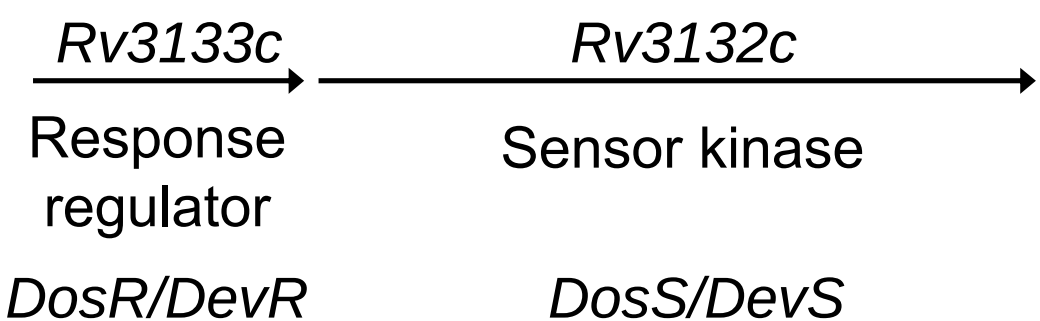


What sets this process in motion?

The initial MTB hypoxic response

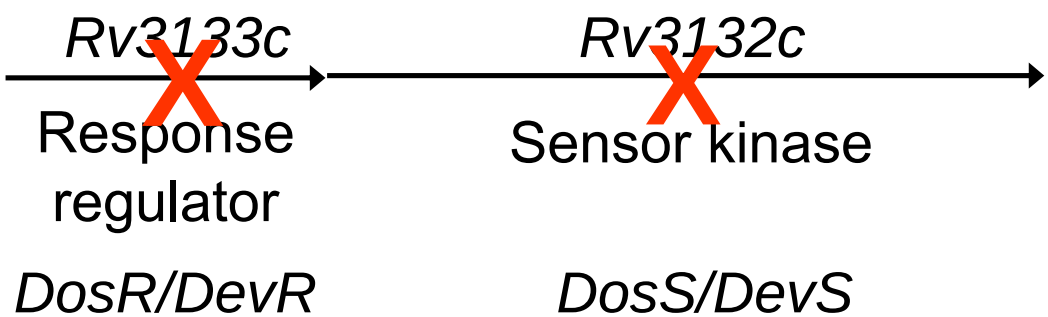
47 genes induced.

One putative transcription factor:



Regulators of the initial MTB hypoxic response

Targeted gene disruptions --



MTB genes induced by hypoxia:

H37Rv	47
H37Rv:Δ*dosR*	8

Is DosR really a transcription factor?

Yes:

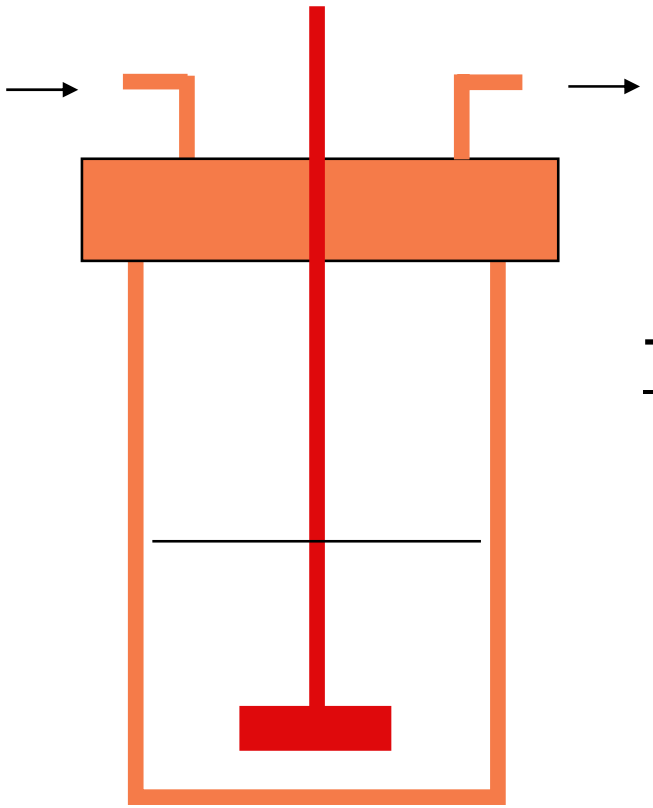
DosR binds upstream of initial hypoxic response genes.

Consensus sequence identified.

Binding is required for induction of initial hypoxic genes.

Park et al., Mol Microbiol, 2003, 48:833-843

MTB and hypoxia -- DosR and beyond



<u>The initial (2 hr) hypoxic response –</u>

cells don't grow; don't die

47 genes induced

controlled by DosR

Is DosR required for long-term persistence?

YES:

Controls the initial hypoxic response.

BCG:Δ*dosR* was attenuated in Wayne model (Boon and Dick, 2002).

Is DosR required for long-term persistence?

YES:

Controls the initial hypoxic response.

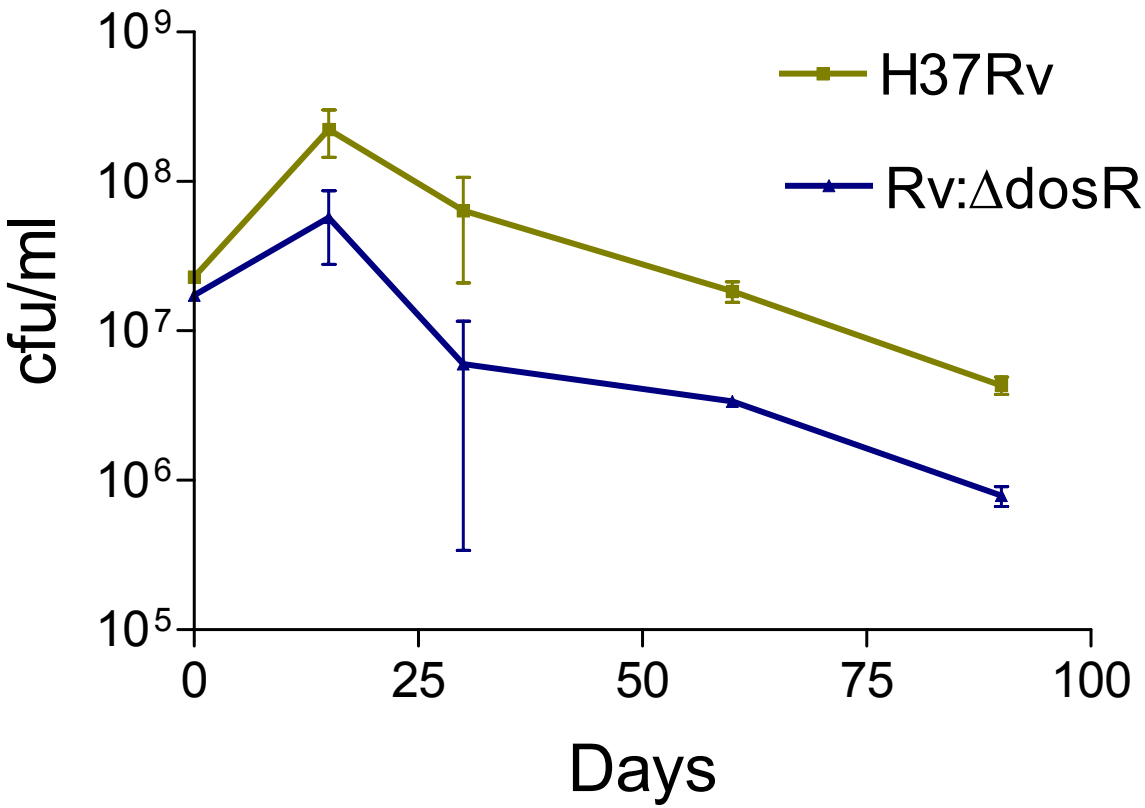
BCG:Δ*dosR* was attenuated in Wayne model (Boon and Dick, 2002).

NO:

DosR regulon is rapidly induced in macrophages and mice, when cells are growing.

Must test phenotypes……..

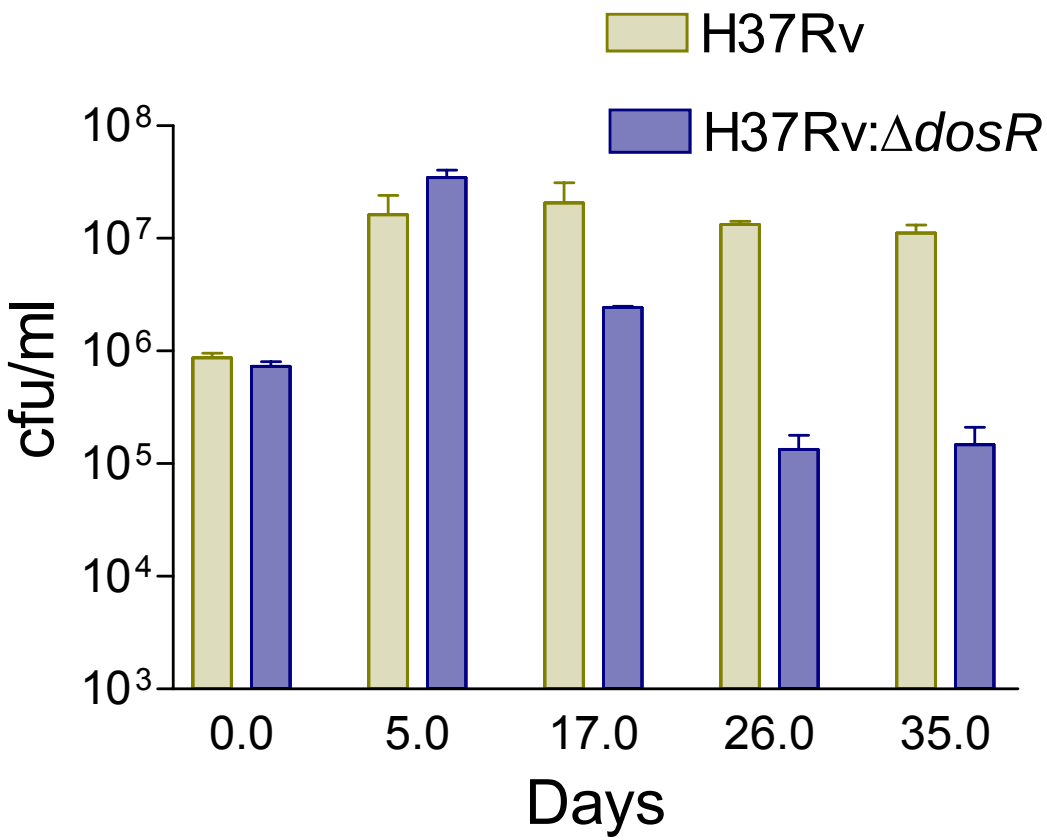
DosR mutants in long-term static culture



Repeated 2X

DosR mutants in Wayne model experiments

Gradual adaptation to hypoxia in vitro

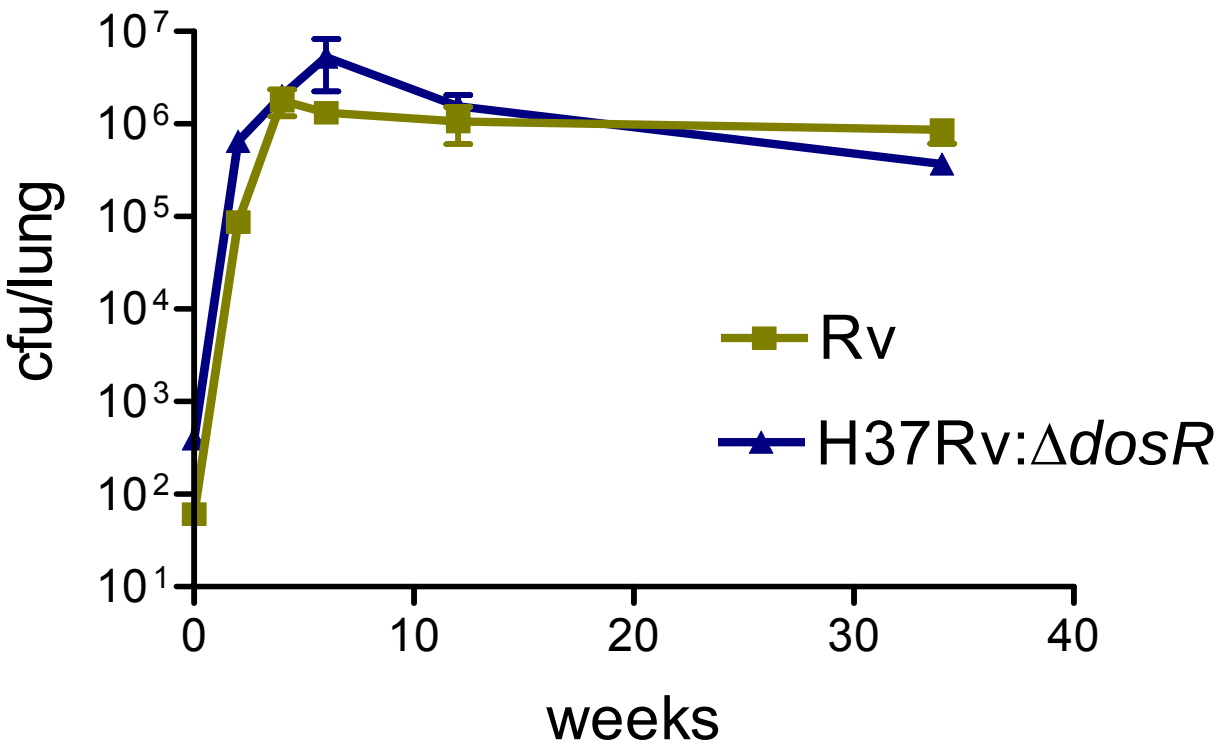


"Repeated" 5X

Range: 8 - 100X diff.

DosR mutants in C57BL/6 mice

Aerosol infection



Repeated 3X

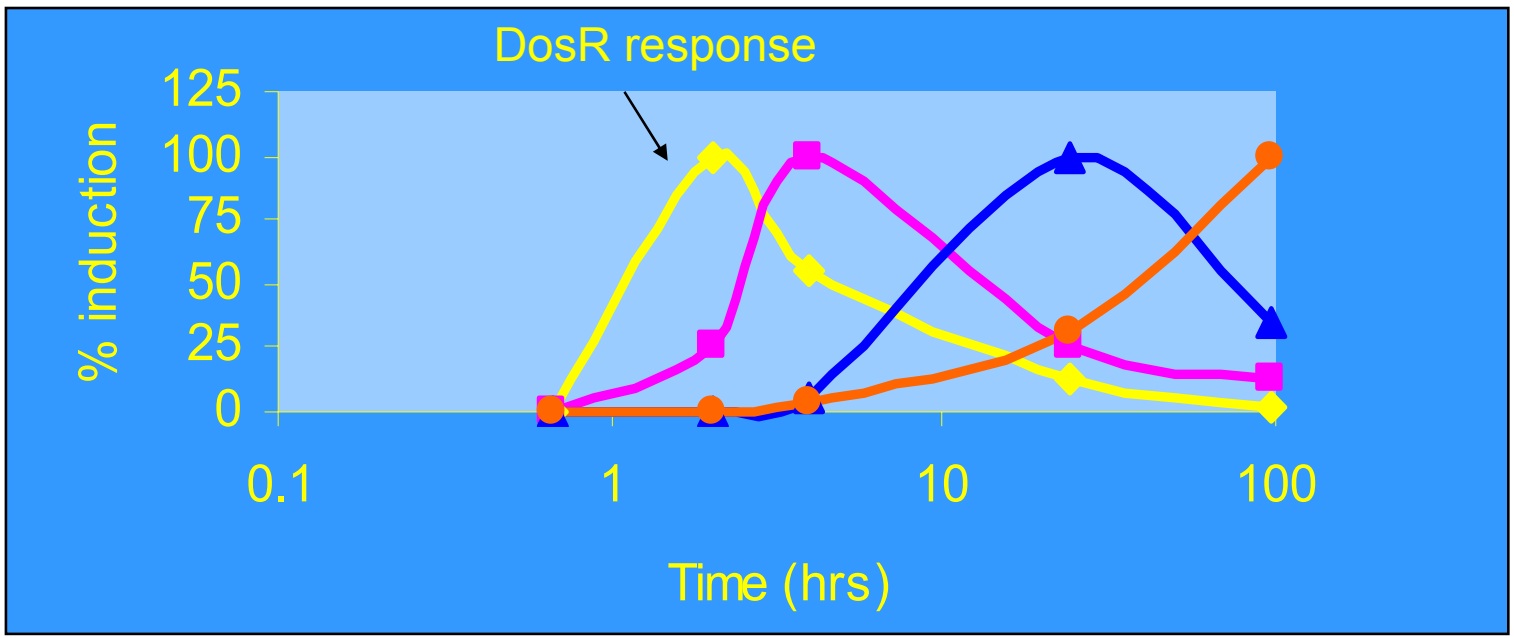
Also in:

DBA/2

C3HeJ

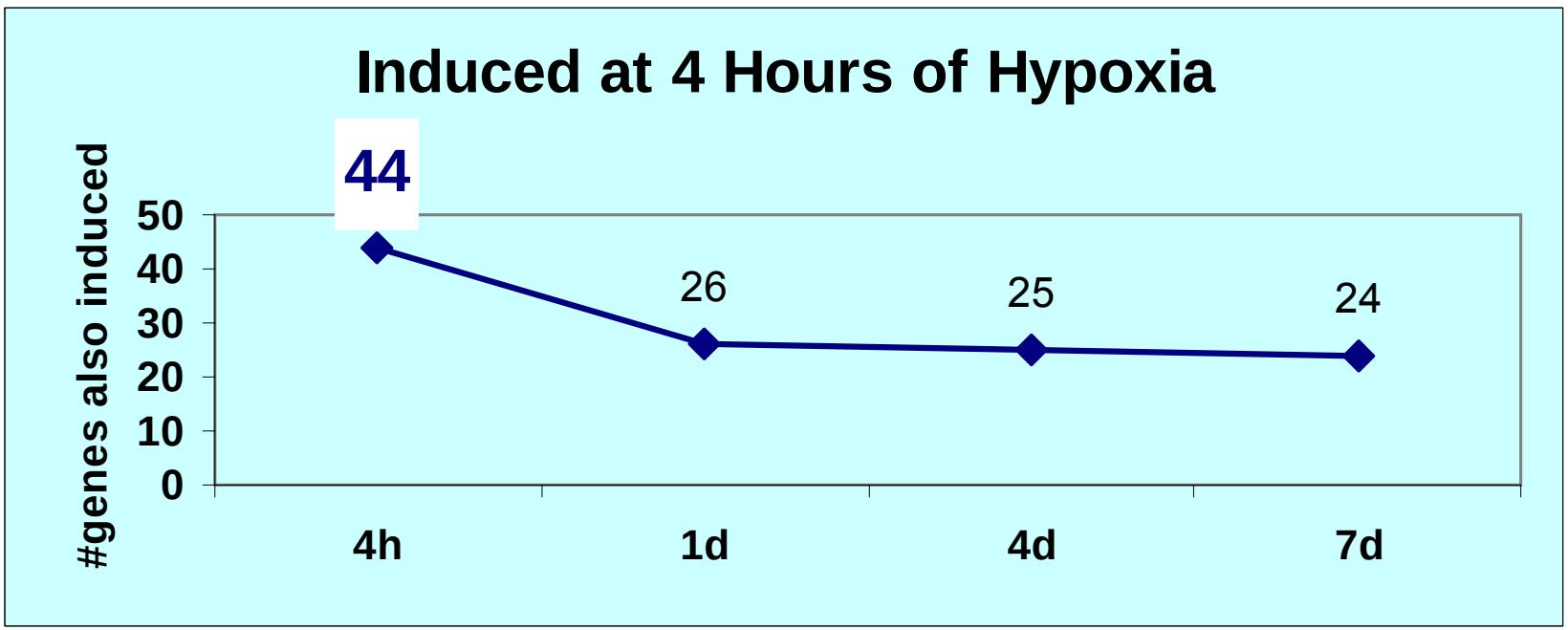
Why isn't the DosR phenotype stronger?

My assumption……



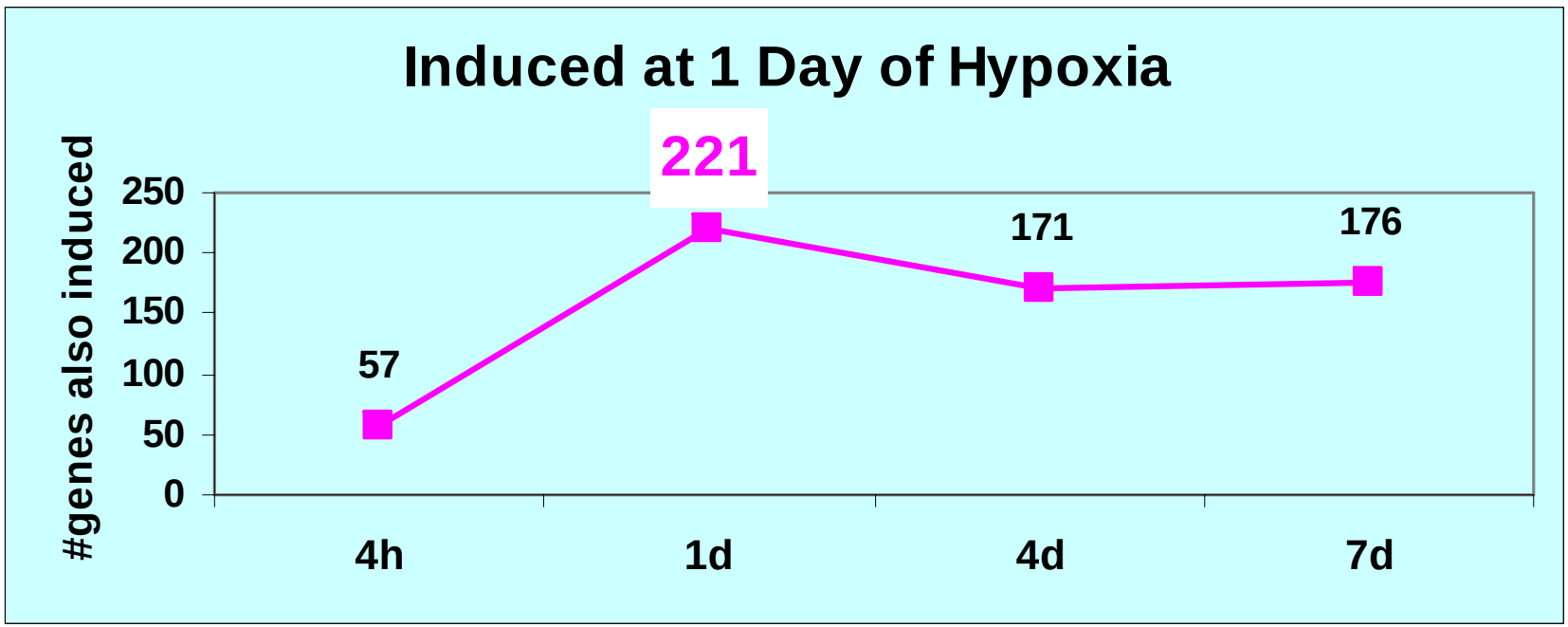
Time to test. Collect RNA, analyze by microarray……..

The MTB hypoxic response – 4 hour genes

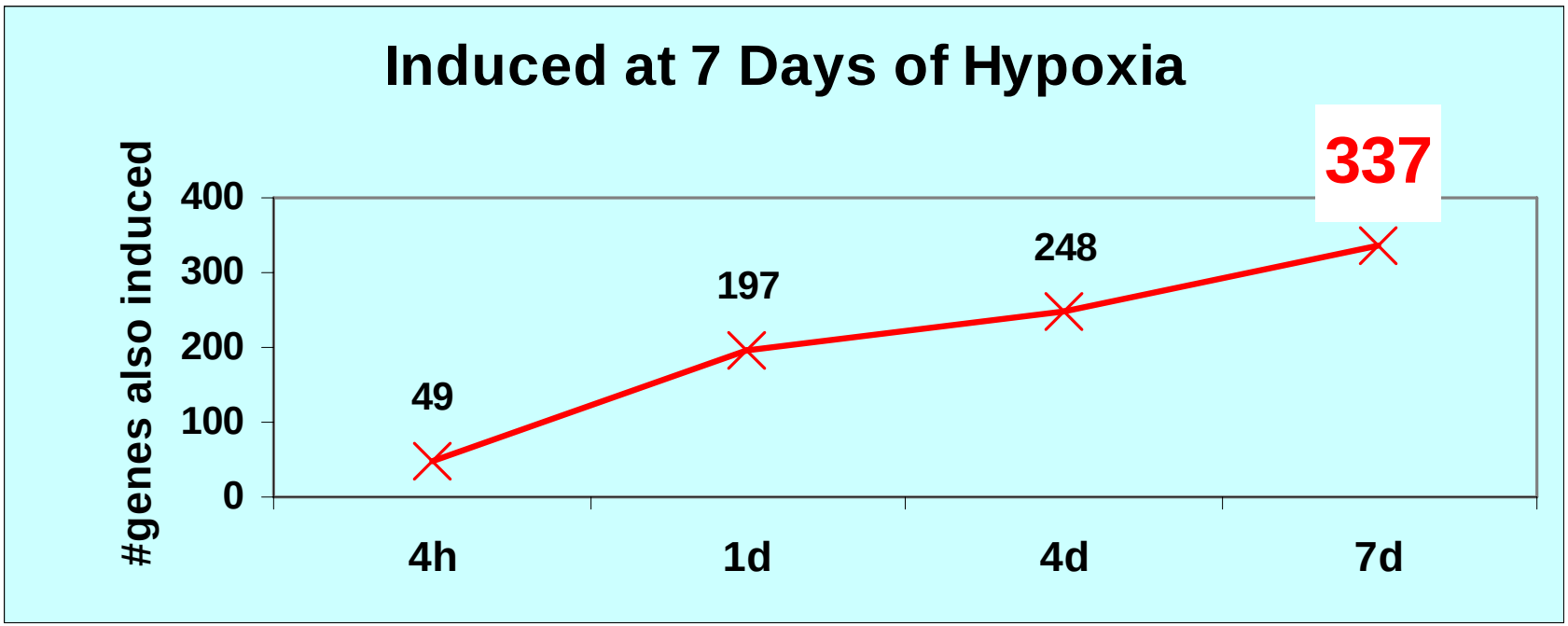


Nearly all are DosR-regulated

The MTB hypoxic response – 1 day genes



The MTB hypoxic response – 7 day genes



Introducing the enduring hypoxic response (EHR)

Defined as all MTB genes induced at 4 AND 7 days hypoxia.

Approx. 250 genes.

Very stably induced.

HMS Endurance

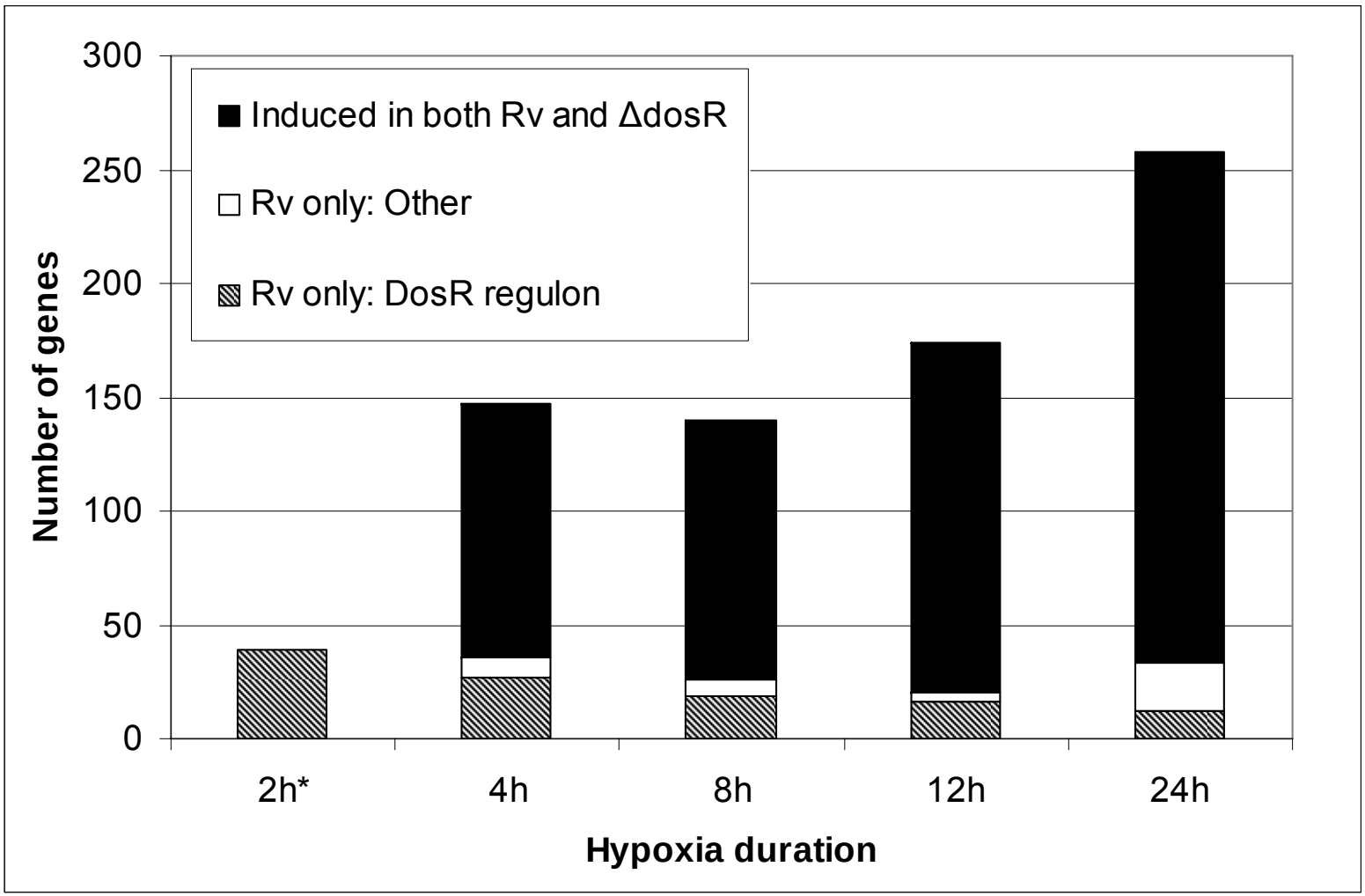
Functional categories within the EHR

Functional categories	EHR #genes	%EHR	%genome
conserved hypotheticals/unknown	80	34.8	31.9
intermediary metabolism and respiration	54	23.5	22.4
cell wall and cell processes	22	9.6	18.8
lipid metabolism	7	3.0	5.9
information pathways	6	2.6	5.8
regulatory proteins	30	13.0	4.8
PE/PPE	13	5.7	4.2
insertion seqs and phages	6	2.6	3.7
virulence, detoxification, adaptation	12	5.2	2.6

The EHR vs. other responses

			EHR: 230 Genes		DosR: 49 genes	
	Condition	**Total**	**Overlap (Chance)**		**Overlap (Chance)**	
Starvation	4 hours ND	170	**23**	(10)	**0**	(2)
	1 day ND	250	**54**	(15)	**0**	(3)
	4 day ND	276	**47**	(16)	**0**	(3)
Wayne model	NRP 4 days	78	**8**	(5)	**42**	(1)
	NRP 20 days	177	**73**	(10)	**33**	(2)
	NRP 80 days	9	**5**	(1)	**0**	(0)
Control stresses	pH 4.8	195	**18**	(11)	**1**	(2)
	Rifampin	375	**15**	(22)	**2**	(5)
	H2O2	199	**6**	(12)	**3**	(2)

The Δ*dosR* mutant and Rv converge to a common transcriptional profile



Hypoxia summary

The MTB response to hypoxia consists of two gene sets:

the initial (DosR) response

the Enduring Hypoxic Response (EHR).

The EHR is stably induced (~10 days).

A defining characteristic of the EHR is the abundance of regulatory proteins.

Disruption of DosR has little impact on the EHR.

Key unanswered questions

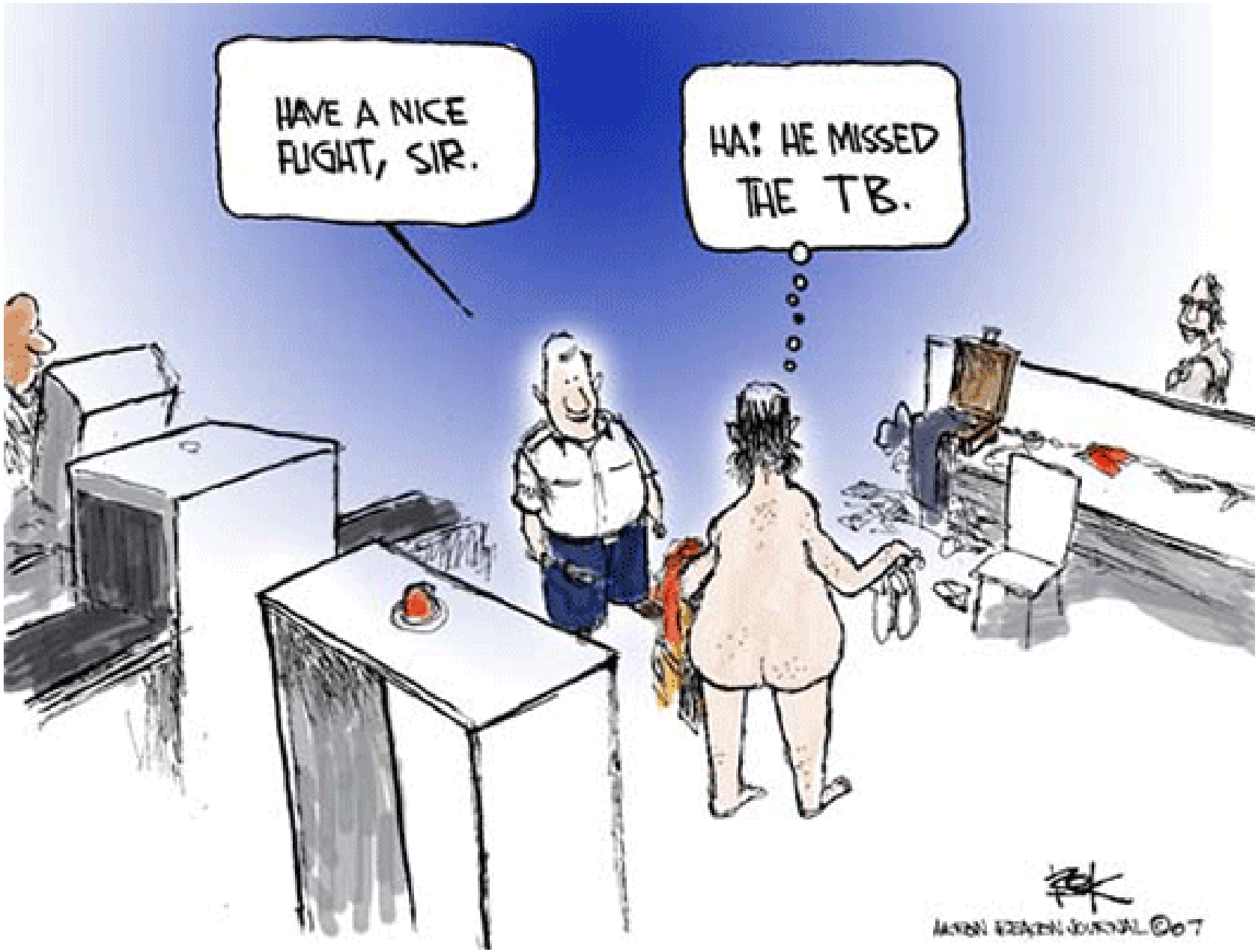
What are the crucial regulators of the EHR?

sigma factors?

What are the crucial metabolic adaptations to hypoxia?

How does hypoxia relate to MTB latency?


THANK YOU!
Tige Rustad
Reiling Liao


HAVE A NICE FLIGHT, SIR.
HA! HE MISSED THE TB.