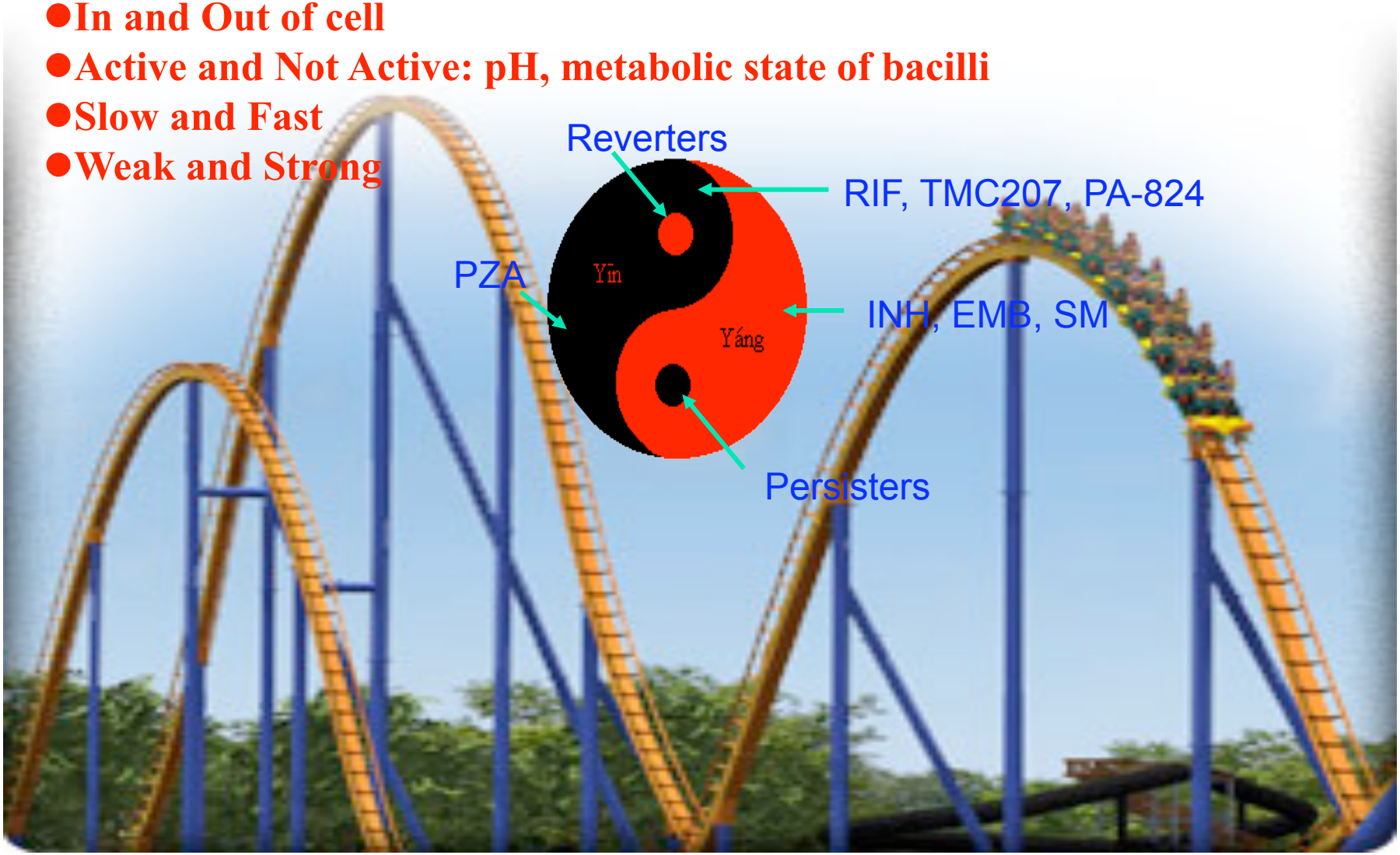
The Roller Coaster of PZA

Ying Zhang, MD, PhD
Department of Molecular Microbiology & Immunology
Bloomberg School of Public Health
Johns Hopkins University
Email: yzhang@jhsph.edu

The Fate of PZA and Interest in PZA

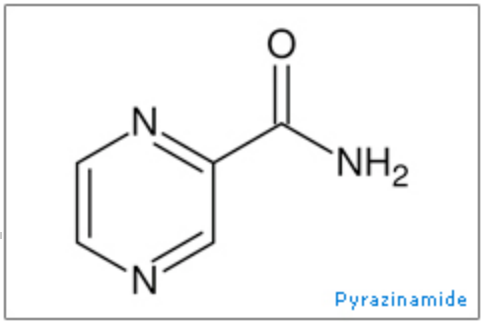
- PZA (prodrug) is dynamic, Yin-Yang (Up and Down) drug, converting to POA
- In and Out of cell
- Active and Not Active: pH, metabolic state of bacilli
- Slow and Fast
- Weak and Strong

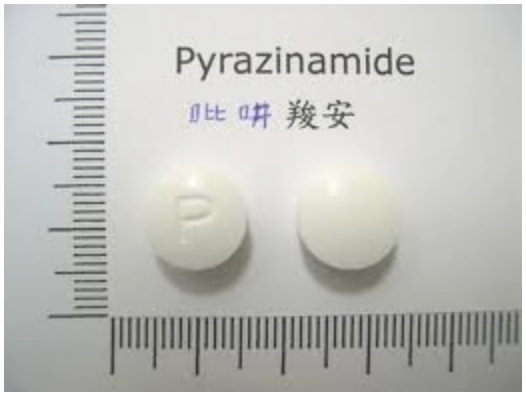



PZA


PYRAZINAMIDE


10
20
30
40


O
N
NH2
N
Pyrazinamide


Pyrazinamide
吡嗪酰胺


Pyrazinamide
Tablets
500mg


吡嗪酰胺片
BIQINXIANANPIAN

PZA: A Unique Drug in Treatment of TB and MDR-TB

- PZA discovery: 1952
- PZA in DOTS: 6 month therapy - The best therapy against TB

-Initial phase (daily, 2 months) with 4 drugs:

INH, RIF, PZA, EMB

-Continuation phase (4 months) with 2 drugs:

INH, RIF

- PZA is used throughout MDR-TB treatment for 18-24 months

Pyrazinamide (PZA):

An unconventional and paradoxical drug

- A most important front-line TB drug, that plays a key role in shortening therapy, because PZA kills persister TB bacilli that are not killed by other TB drugs

- Despite its powerful sterilizing activity in shortening therapy, curiously, no activity against TB bacilli in vitro in culture medium when bacteria are growing

- PZA is opposite to common antibiotics

Special Bacterial Populations Theory (Mitchison Hypothesis)

High

Speed of bacterial growth

Low

INH (RIF, SM, EMB)

A. Continuous growth

RIF

PZA

B. Spurts of metabolism

C. Acid inhibition

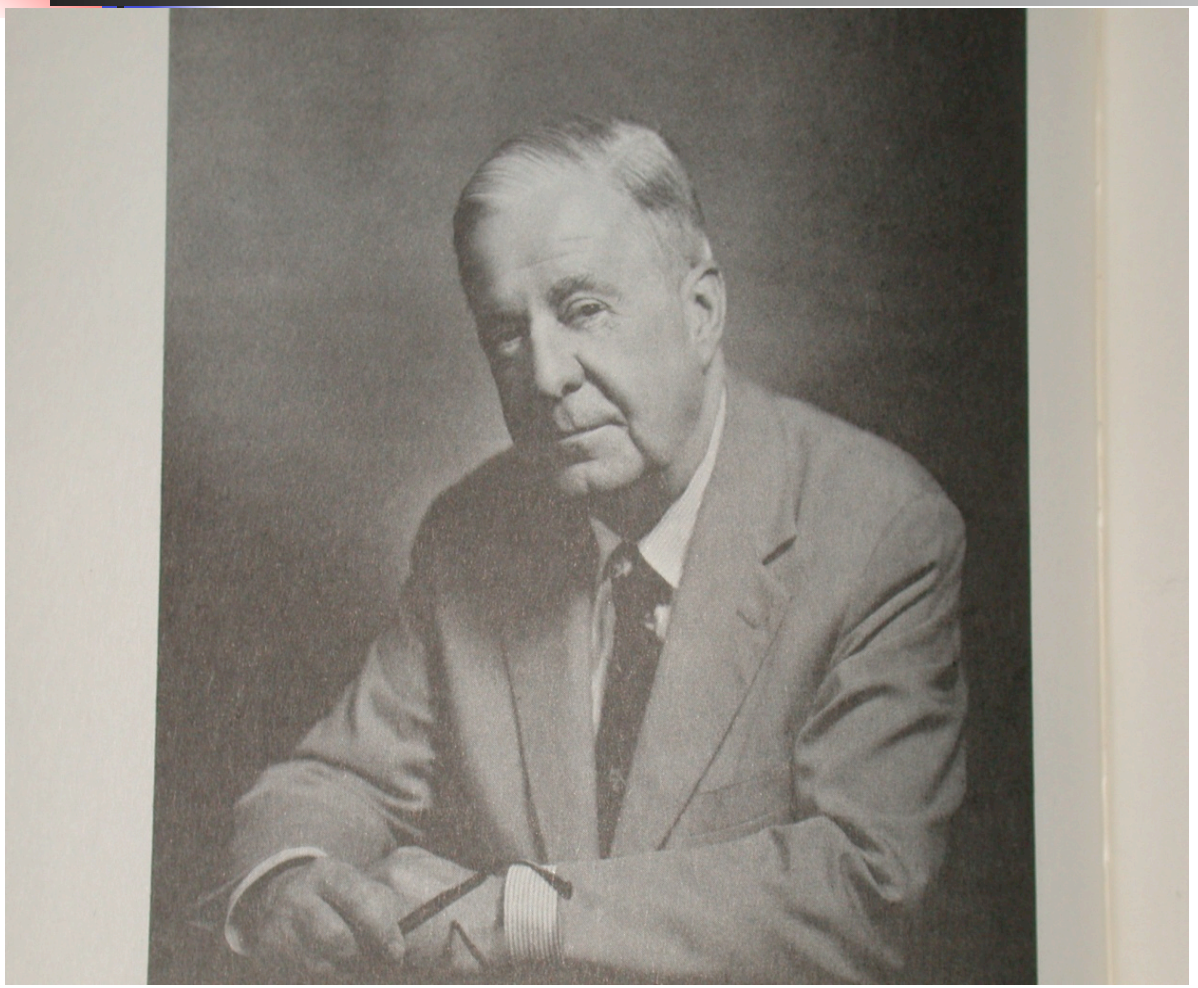
Semi-dormant

D. Dormant

Paradoxical Features of PZA

- PZA is active at acidic pH (pH 5.5) (McDermott 1954)
- MIC is high = 50-100 μg/ml at acid pH 5.5-6.0, and kills MTB slowly
- PZA kills old, dormant bacilli more effectively than actively growing bacilli (Zhang et al., 2002)
- PZA kills non-replicating persisters more effectively under hypoxic/anaerobic conditions (Wade and Zhang, 2004), by energy inhibitors (Zhang et al., 2003 JAC)
- In vivo (mice or humans), it has impressive sterilizing activity and is involved shortening the therapy (McDermott 1956)
- EBA studies in humans: INH has high EBA in first 3 days, PZA has poor in first 2 weeks

Walsh McDermott (1909-1981) -
Founding father of IOM, National Academies

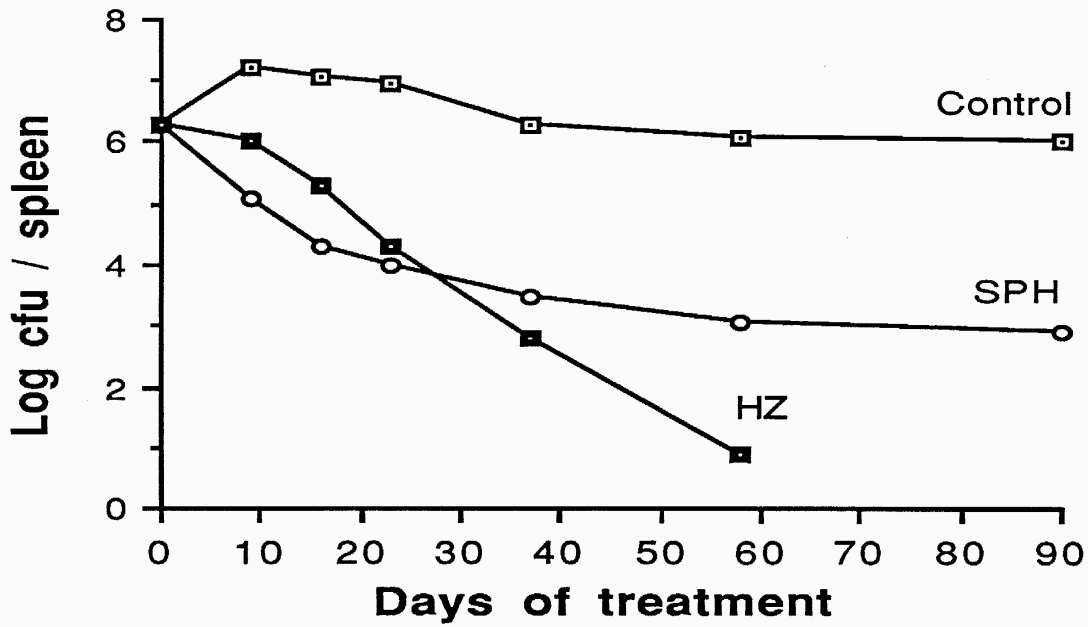


Clinical evaluation of INH- (Lasker Award, 1955)

Cornell mouse model of TB Persistence

Work on PZA:
(a) acid pH requirement
(b) PZA-resistant TB lose PZase
(c) unique sterilizing activity of PZA in mice

PZA achieves high sterilizing activity with INH - Basis for SCC



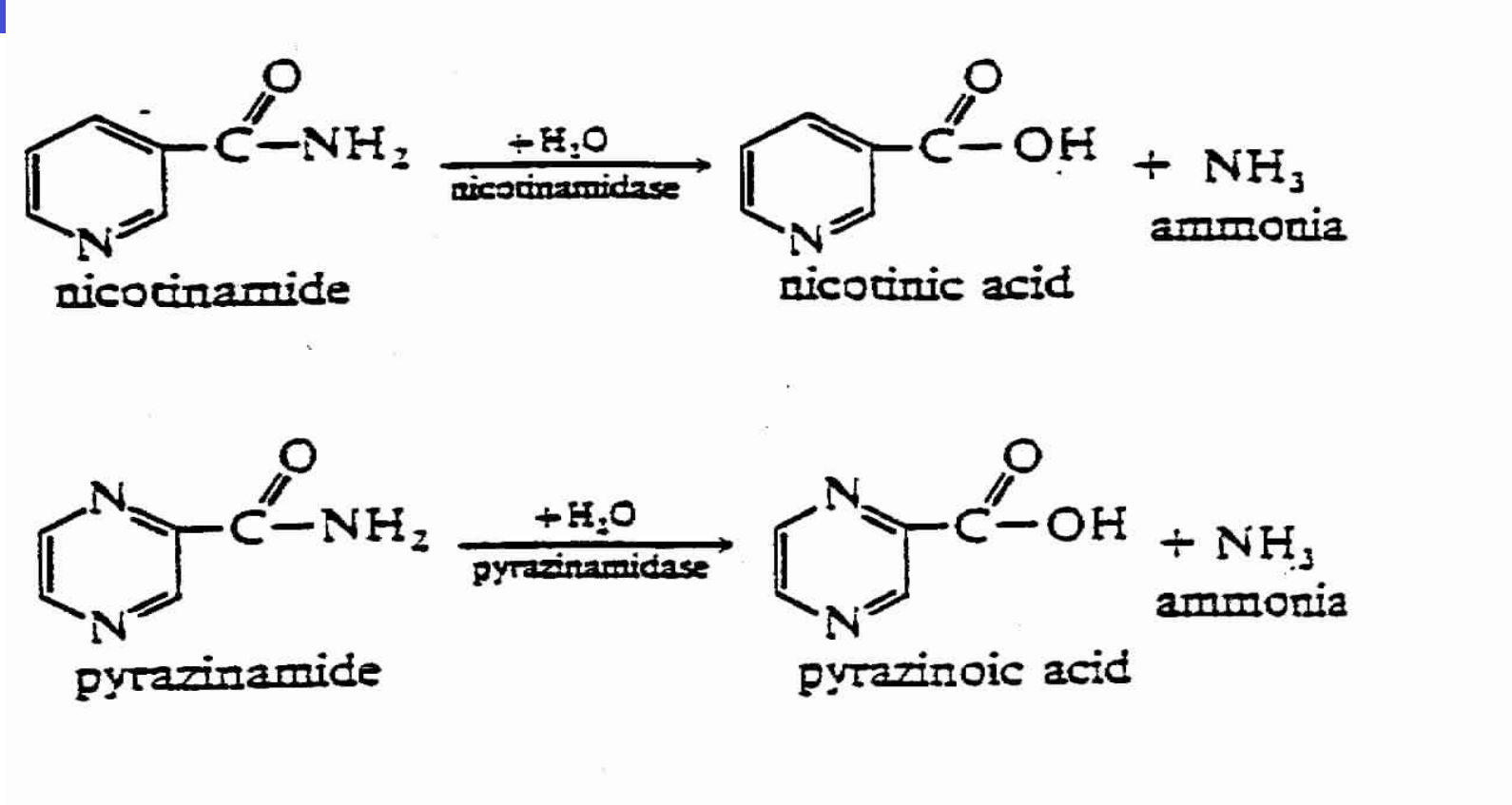
From McCune R M, Tompsett R, McDermott W J Exp Med 1956; 104: 763-802.


FINISH
STUDIOS, INC

History of PZA

- Unconventional discovery: In 1944, Chorine found nicotinamide (vitamin B3) had antimycobacterial activity in animal models-> in 1948, Lederle Lab made analogs of nicotinamide -> PZA
- Used in clinical treatment in 1952 (same year as INH), mainly used as a second-line drug for drug-resistant cases or relapse because of liver toxicity due to high dose and long term use
- Inspired by McDermott's studies, British MRC conducted clinical trials late 1970s and early 80s, PZA was found to shorten the therapy from previously 9-12 months to 6 months -> used as first-line drug -> Basis for modern short-course TB therapy

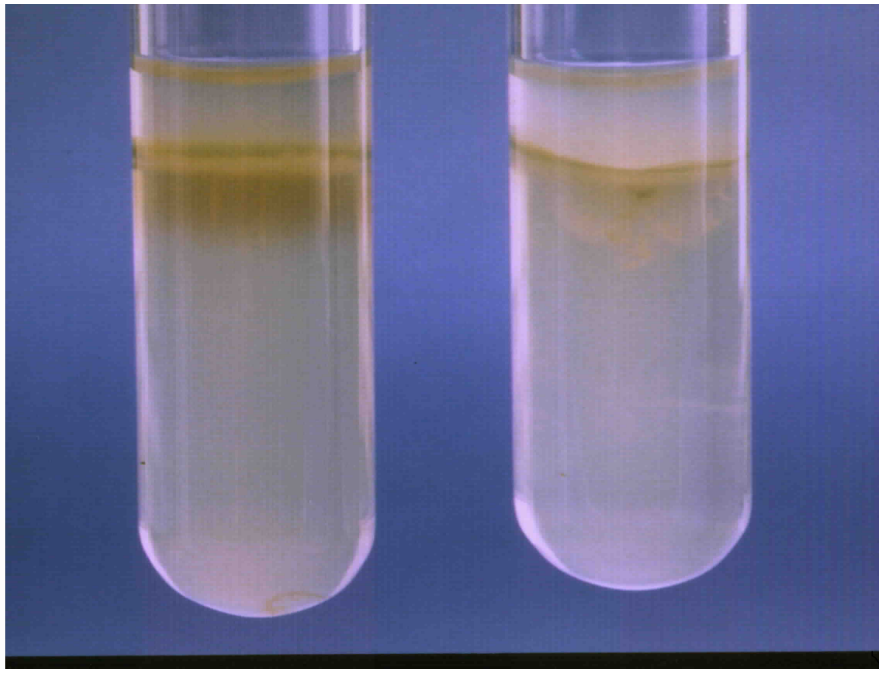
PZA Resistance



In 1967, McDermott and colleagues showed that PZA-resistant strains lose pyrazinamidase/nicotinamidase enzyme activity

Cloning of TB *pncA* gene

- Cloning of pyrazinamidase gene (*pncA*) and found mutation in *pncA* gene is the major mechanism of PZA resistance (Scorpio and Zhang, 1996, Nature Medicine, 2:662-667)



Mechanism of PZA Resistance

- Mutations in *pncA* gene are the major mechanism of PZA resistance: 72-97%(85%), small number with low level R no *pncA* mutations

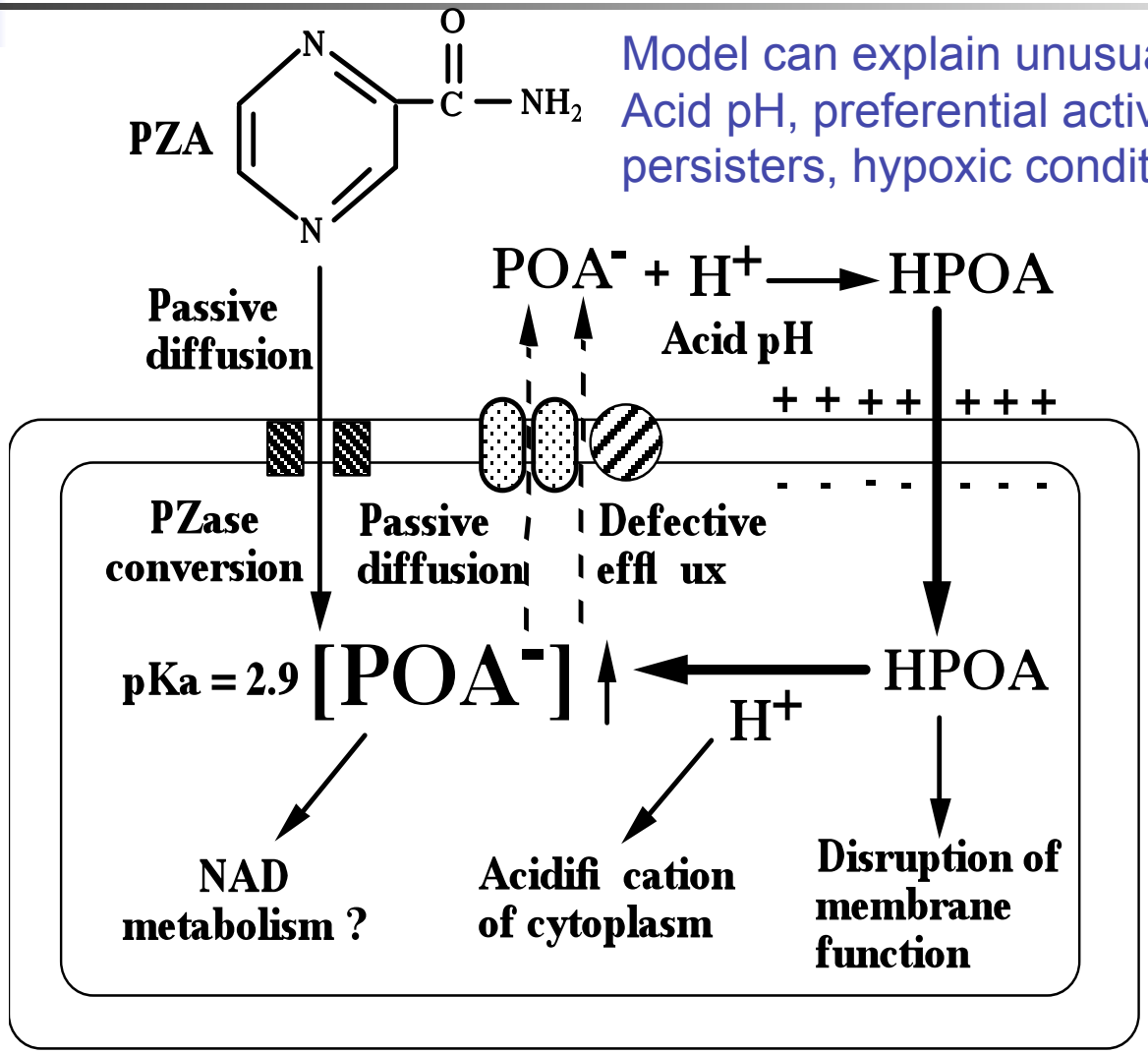
- *M. bovis* strains including BCG are naturally resistant to PZA and lack PZase enzyme, due to a single characteristic point mutation in *pncA* gene, at nt. 169 from “C” to “G”, causing His57Asp

- Rapid differentiation of *M. bovis* from MTB strains based on detecting the “C” to “G” point mutation in the *pncA* gene

How does PZA work?

- The role of acid pH (Zhang et al., 1999):
- Henderson-Hasselbalch equation showing the relation between pH and PZA activity: (Zhang et al., 2002)
- PZA kills old, dormant bacilli more effectively than actively growing bacilli (Zhang et al., 2002)
- POA disrupt membrane potential and inhibits transport (Zhang et al., 2003)
- PZA kills non-replicating persisters more effectively under hypoxic/anaerobic conditions (Wade and Zhang, 2004)
- PZA activity is enhanced by energy inhibitors (Zhang et al., 2003), iron (Somoskovi et al., 2004), UV, weak acids (Wade et al., 2006; Gu et al., 2008)

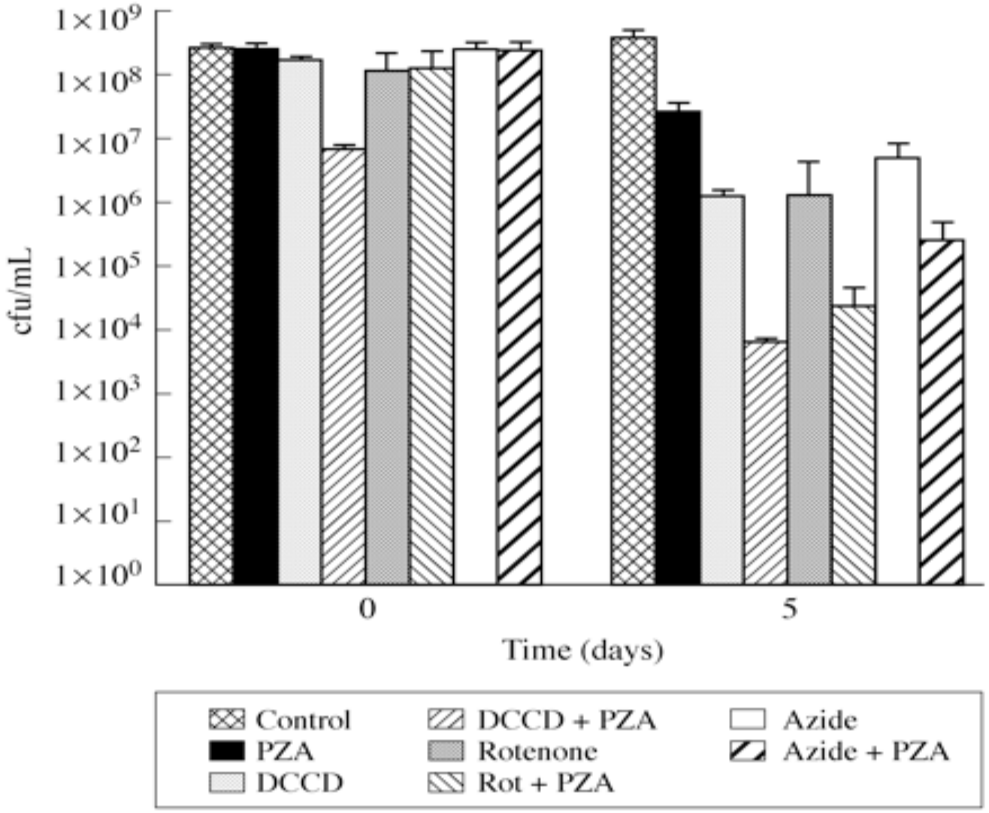
Mode of Action of PZA (2003)



Model can explain unusual properties of PZA: Acid pH, preferential activity for non-replicating persisters, hypoxic conditions

Enhanced PZA activity by energy inhibitors

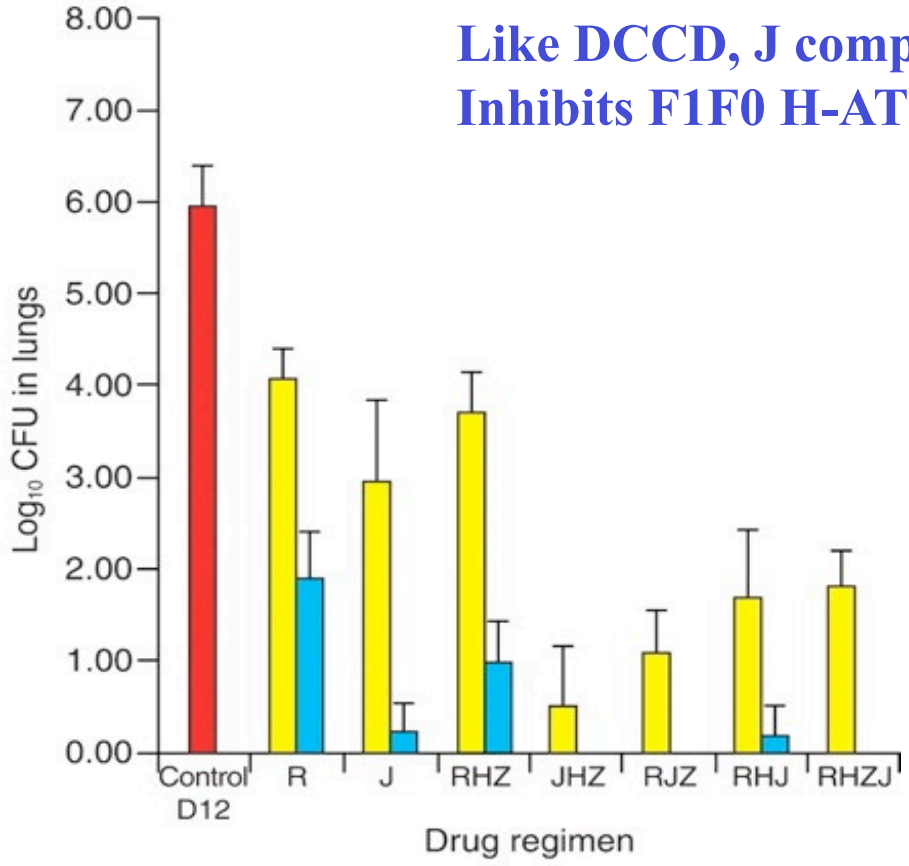
Zhang et al. J Antimicrob Chemother 2003, 52:790-5



PZA=100 μg/ml; 5 day incubation at pH5.5

Synergy Between Diarylquinoline (J) and PZA (Andries et al., 2005, Science, 307: 223-7)

Like DCCD, J compound Inhibits F1F0 H-ATPase



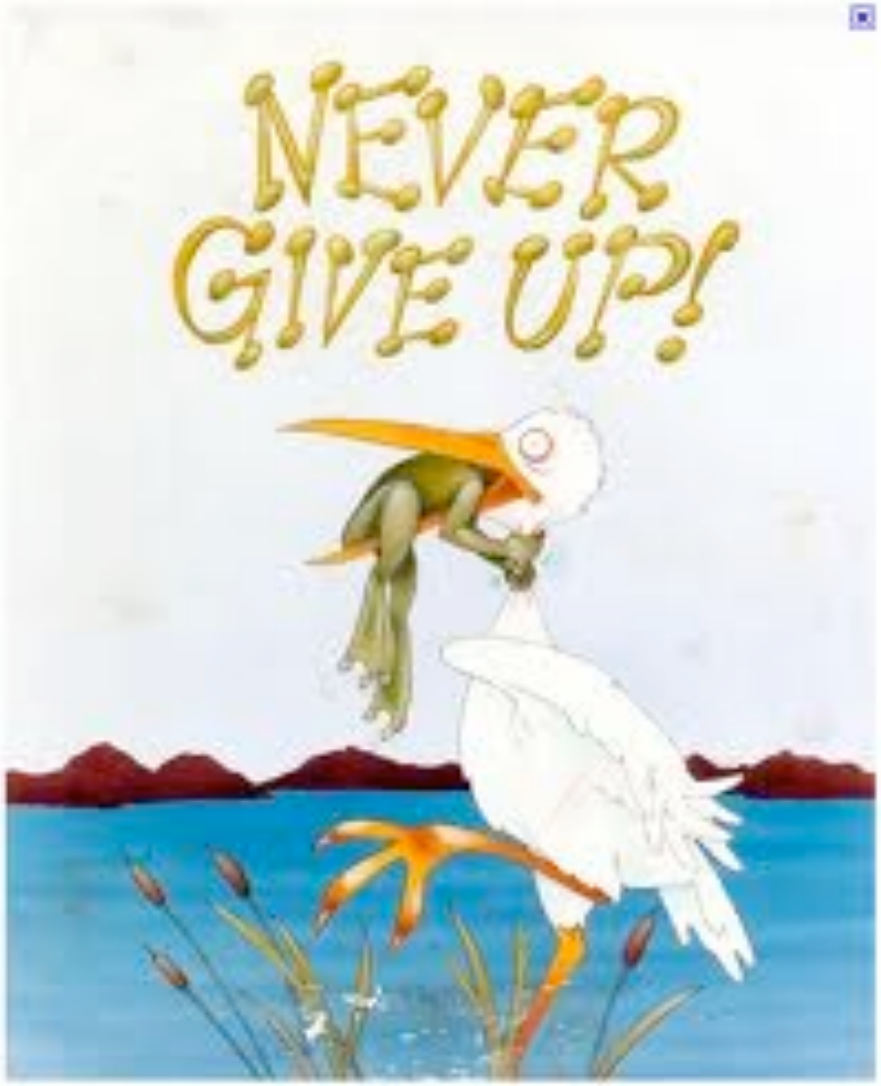
What is the target of PZA?

- Fas-I was proposed as a target of PZA by Zimhony et al., (Nat Med, 2000, 6: 1043-7)
- However, Boshoff et al. showed that Fas-I is the target of 5-Cl-PZA, but not the target of PZA (J. Bacteriol. 2002, 184: 2167-72)


HARD
WORK
AHEAD


WeirdBum.com


FAIL.

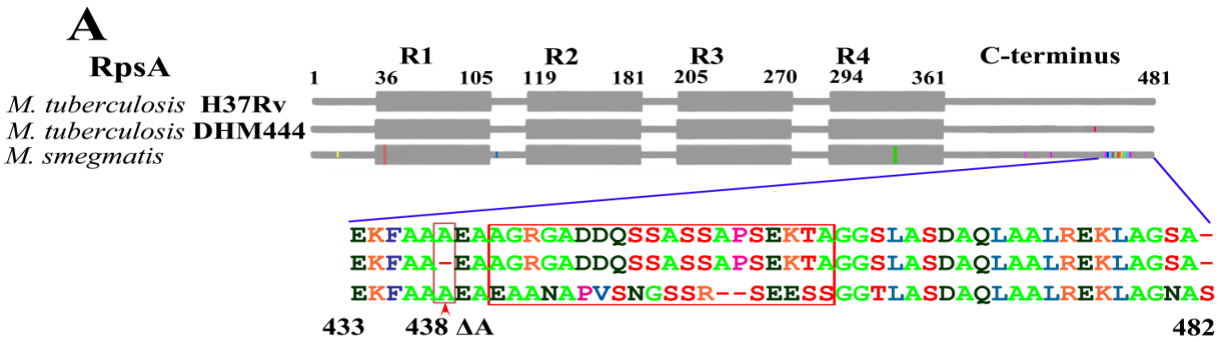

NEVER
GIVE UP!

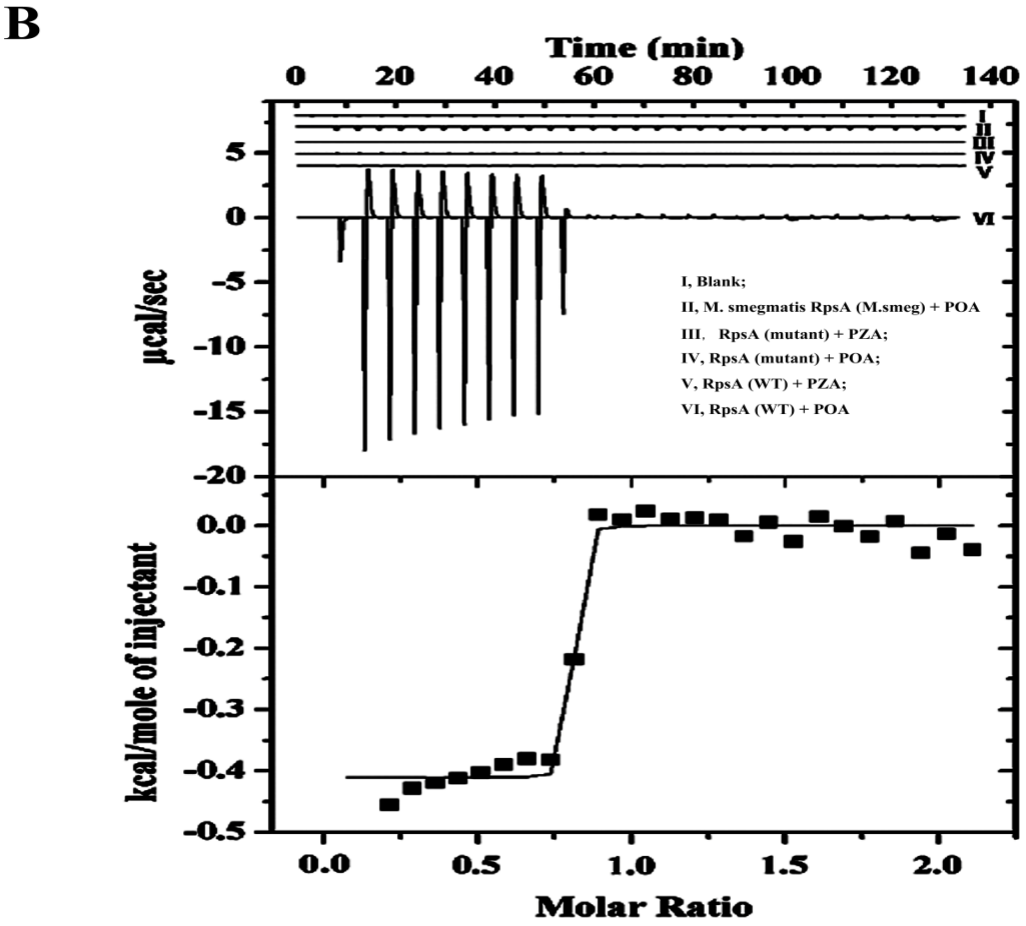
A New Target of PZA: RpsA

We identified a new target of PZA RpsA that binds to POA

Overexpression of RpsA conferred 5-fold PZA resistance in MTB from 100 to 500 μg/ml PZA

A low level PZA-resistant *M. tuberculosis* DHM444 (MIC 200-300 μg/ml PZA) without *pncA* mutations (Scorpio 1997), contained a 3-bp deletion (ΔGCC) alanine missing in the C-terminus of RpsA


A
RpsA
R1
R2
R3
R4
C-terminus
1
36
105
119
181
205
270
294
361
481
M. tuberculosis H37Rv
M. tuberculosis DHM444
M. smegmatis
EKFAAAEAAGRGADDQSSASSAPSEKTAGGSLASDAQLAALREKLAGSA-
EKFAA-EAAGRGADDQSSASSAPSEKTAGGSLASDAQLAALREKLAGSA-
EKFAAAEAEAANAPVSNGSSR--SEESSGGTLASDAQLAALREKLAGNAS
433
438 ΔA
482

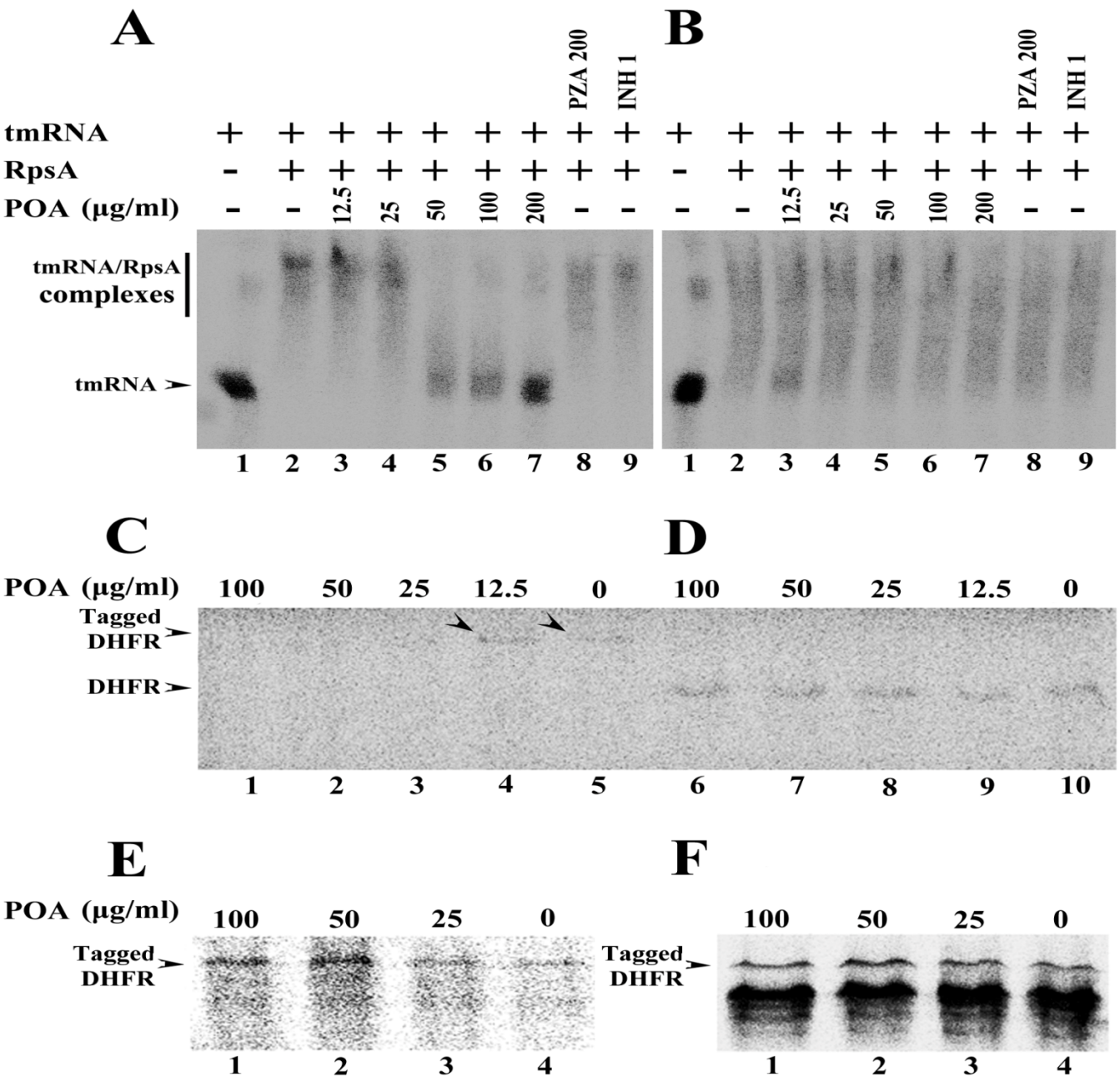

B
Time (min)
0
20
40
60
80
100
120
140
I
II
III
IV
V
VI
µcal/sec
5
0
-5
-10
-15
-20
I, Blank;
II, M. smegmatis RpsA (M.smeg) + POA
III, RpsA (mutant) + PZA;
IV, RpsA (mutant) + POA;
V, RpsA (WT) + PZA;
VI, RpsA (WT) + POA
kcal/mole of injectant
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
0.0
0.5
1.0
1.5
2.0
Molar Ratio

Trans-translation and PZA

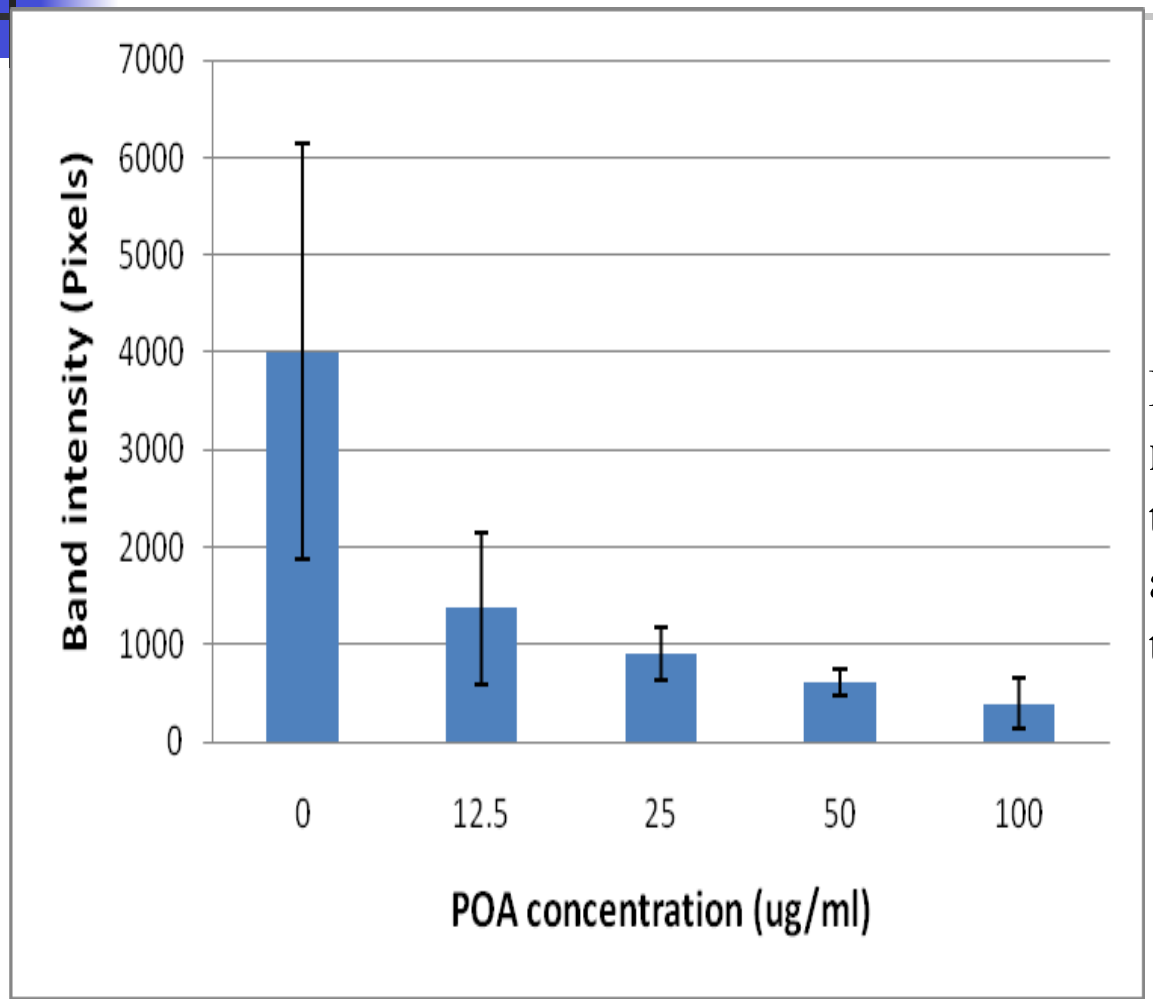
Trans-translation rescues ribosomes stalled on defective mRNAs and adds a specific peptide tag sequence encoded by tmRNA to aberrant polypeptides for degradation by proteases.

Trans-translation is dispensable during active growth but becomes important under stress conditions in managing stalled ribosomes or damaged mRNA and proteins, involved in stress survival and virulence

RpsA binds tmRNA and facilitates trans-translation mediated by a complex of tmRNA, SmpB, Ef-Tu, RpsA

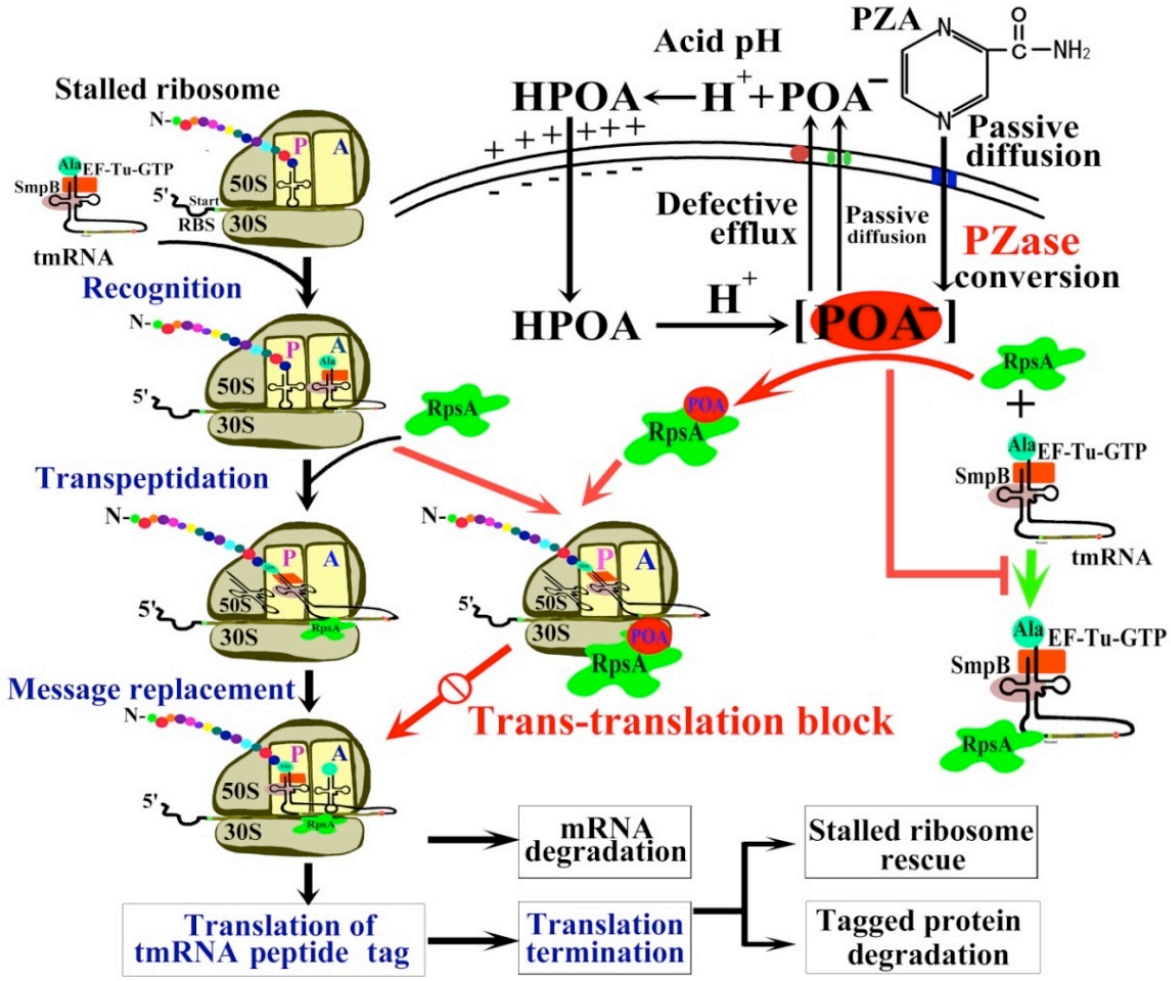

A
B
tmRNA
RpsA
POA (µg/ml)
PZA 200
INH 1
12.5
25
50
100
200
tmRNA/RpsA complexes
tmRNA
1 2 3 4 5 6 7 8 9
C
D
POA (µg/ml) 100 50 25 12.5 0 100 50 25 12.5 0
Tagged DHFR
DHFR
1 2 3 4 5 6 7 8 9 10
E
F
POA (µg/ml) 100 50 25 0
Tagged DHFR
1 2 3 4

POA inhibits trans-translation in MTB in a concentration-dependent manner



Implication for developing new drugs that target trans-translation, to avoid acid pH so it can be used throughout therapy

A Revised New Model of PZA



Molecular Methods for Detecting Mutations in Drug Resistance Genes

- PCR, followed by DNA sequencing, PCR-RFLP (HapII-katG 315), SSCP, heteroduplex formation, WAVE-DHPLC, molecular beacons, hybridization (Line-Probe assay) tests in microarray and macroarray, real-time PCR (TaqMan)
- **Line-Probe assays (Hain Lifescience GenoType MTBDRplus)** currently being evaluated in the field with promising results
- **Xpert MTB/RIF TB test Cepheid** (New England Journal Medicine, 2010) 1,730 patients with suspected drug-sensitive or multidrug-resistant pulmonary TB, identified 98 percent of all confirmed TB cases and 98 percent of patients with RIF-resistant bacteria in less than two hours.
- Line-probe assay and Xpert will not work for PZA resistance detection

Rapid Detection of PZA Resistance: *pncA* sequencing for mutations

- PZA susceptibility testing is not performed, problem of DST at acid pH, survey no PZA resistance data
- Acid pH inhibits growth (25-30% strains)
- BACTEC PZA test (Gold standard) pH 6.0, MIC cutoff of 100 μg/ml has false resistance problem, too slow (2 weeks), expensive
- We propose to sequence *pncA* gene for all drug-resistant TB strains (Mayo Clinic in US)

  -good correlation w *pncA* mutations and PZA resistance (>85%)

  -current PZA susceptibility testing is time-consuming, unreliable and expensive

PZA and New TB Drug Candidates – Indispensable, Synergy

FDA approved drugs:

-New rifamycin: rifapentine approved by FDA in 1998; Rifapentine at higher dose plus moxifloxacin and PZA can shorten TB therapy to 2 months in mice (PLoS Med. 2007;4:e344): Phase II

-Linezolid: Phase I and II trials

Drug candidates under clinical development:

-New fluoroquinolones (Bayer): moxifloxacin/gatifloxacin, Phase II, III

-Diarylquinoline (TMC207) (Tibotec, Johnson & Johnson): TMC207 showing good activity in mice and shorten treatment with INH+PZA to 2 month-> Phase II trial (MDR-TB and drug susceptible TB)

-Nitroimidazoles: Phase II trials

(a) Nitroimidazolepyran (PA-824) (TB Alliance): PA-824+Moxi+PZA more effective than current therapy INH+RIF+PZA in mice

(b) OPC-67683 (Otsuka), a nitro-dihydro-imidazooxazole derivative, OPC +RIF+PZA shorten therapy to 2 months in mice

-Ethambutol analog, SQ-109 (Sequella), Phase I trial

Learn from PZA: Synergy

Power of Synergy through Collaborations and Partnerships

The Fate of PZA and Interest in PZA

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Thank You!
Libaiwa