Mechanisms of Action and Resistance to Pyrazinamide – a 20-Year Perspective

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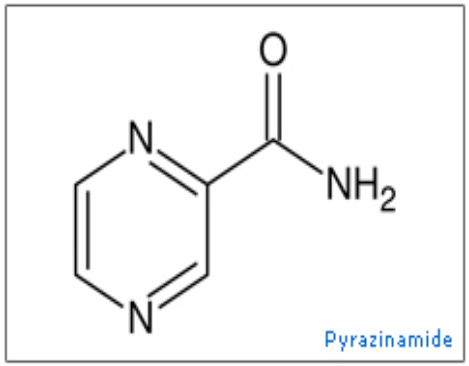
Paradoxes and Fate of PZA:

- PZA (prodrug) is Yin-Yang drug, converting to POA, dynamic
- In and Out of Cell
- Non-Active and Active: pH, metabolic state
- Simplest and Complexest
- Slow and Fast
- Weak (MIC) and Strong
- Worst and Best
- Down and Up: 2nd line → 1st
- Hate and Love

Pyrazinamide, PZA, Z: A Brief History



- Dalmer and Walter synthesized PZA 1936 (1934)
- Chorine: nicotinamide (NAm) 1945
- Kushner, McKenzie: analogs of NAm → PZA 1952
- McDermott: unique sterilizing activity 1956
- BMRC: shortening TB therapy (E Africa) 1972
- WHO: HRZE 6 months 1995; Z for MDR-TB 24 months 2010

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

- PZA in DOTS: 6 month therapy - The best TB therapy

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

INH, RIF, PZA, EMB

-Continuation phase (4 months) with 2 drugs:

INH, RIF

Most important sterilizing drug, a key role in shortening therapy from 9-12 months to 6 months

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

PZA: Unconventional and Paradoxical

- PZA not active at neutral pH, active at acid pH (McDermott, 1954)
- MIC is high = 50-100 μg/ml (pH5.5-6.0), poor activity for growing bacilli
- PZA kills non-growing persisters (Zhang et al., 2002), under hypoxic/anaerobic conditions (Wade and Zhang, 2004), more active against RIF-persisters (Hu, Coates and Mitchison, 2006)
- In vivo, impressive sterilizing activity →shortening therapy in mice (McDermott 1956)
- EBA studies in humans and in mice: INH has high EBA in first 2 days, PZA low EBA in first 2 weeks (Jindani and Mitchison), BUT in combination PZA kills persisters even during early stage (Grosset et al., 2012, PNAS)

- PZA is opposite to common antibiotics

Why is PZA Important?
PZA Kills Persisters and Shorten Therapy

Slide 6

l1 why can stem cells? The failure of cancer chemotherapy could be explain as dendelion phenomenon.
l, 2/3/2007

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

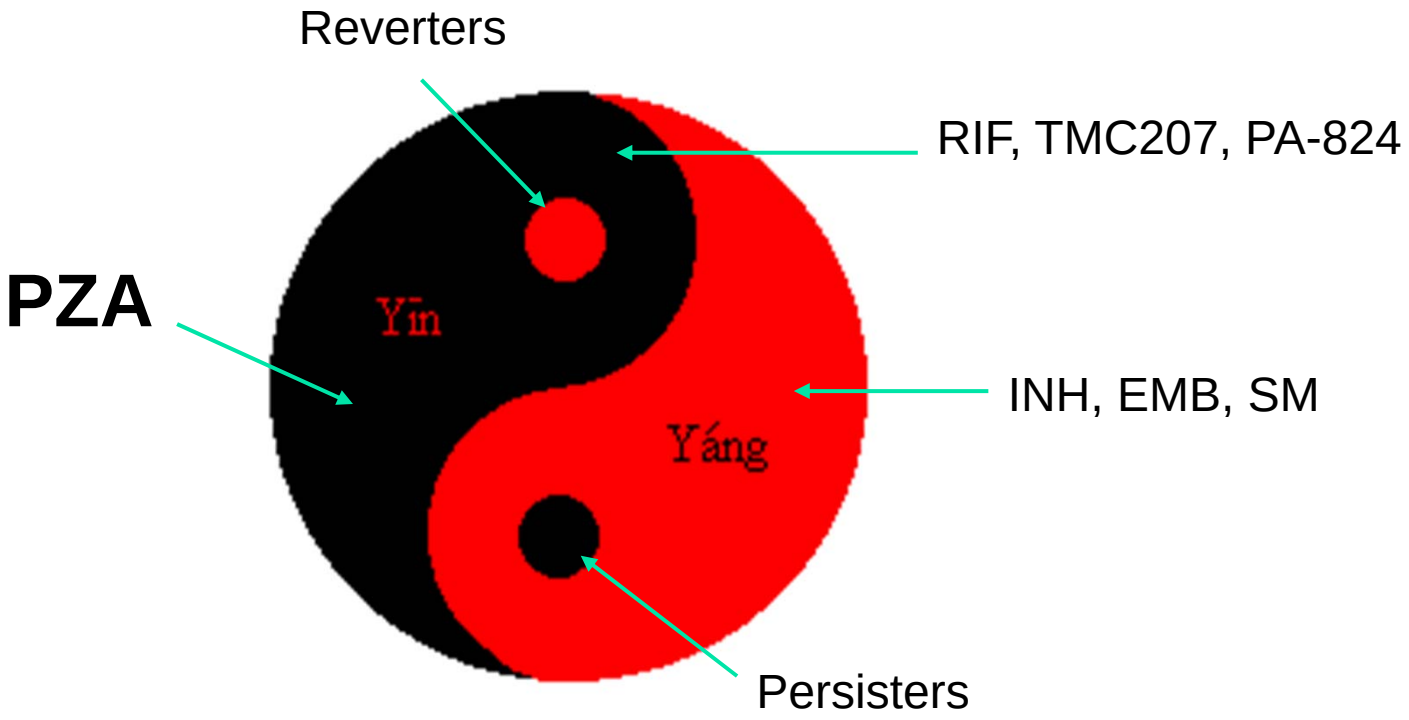
D. Dormant

Yin-Yang Model: Effect of Drugs

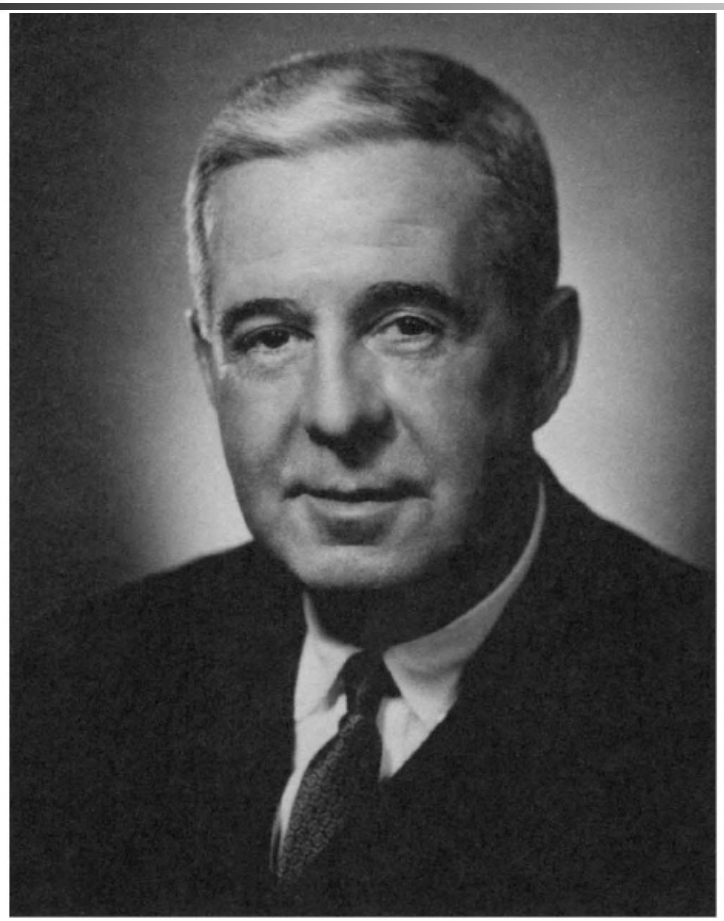
(Y. Zhang, Clin Pharmacol Ther. 2007; 82:595-600)

Day and Night
Matter and Dark Matter
Body and Mind
Conscious and Subconscious

- Bacterial populations
- Genetic vs phenotypic resistance
- LTBI vs active TB
- Explains current TB therapy
- Explains INH prophylaxis for LTBI



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

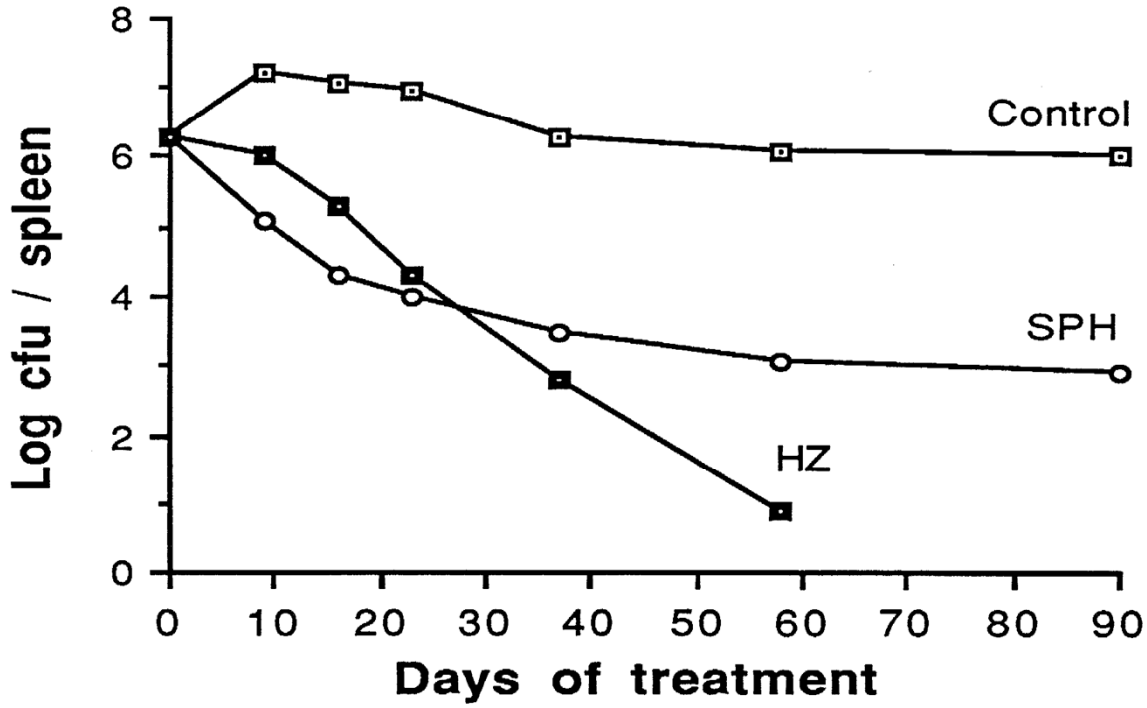


Clinical evaluation of INH- (Lasker Award, 1955)

Microbial persistence:
Cornell model of TB persistence

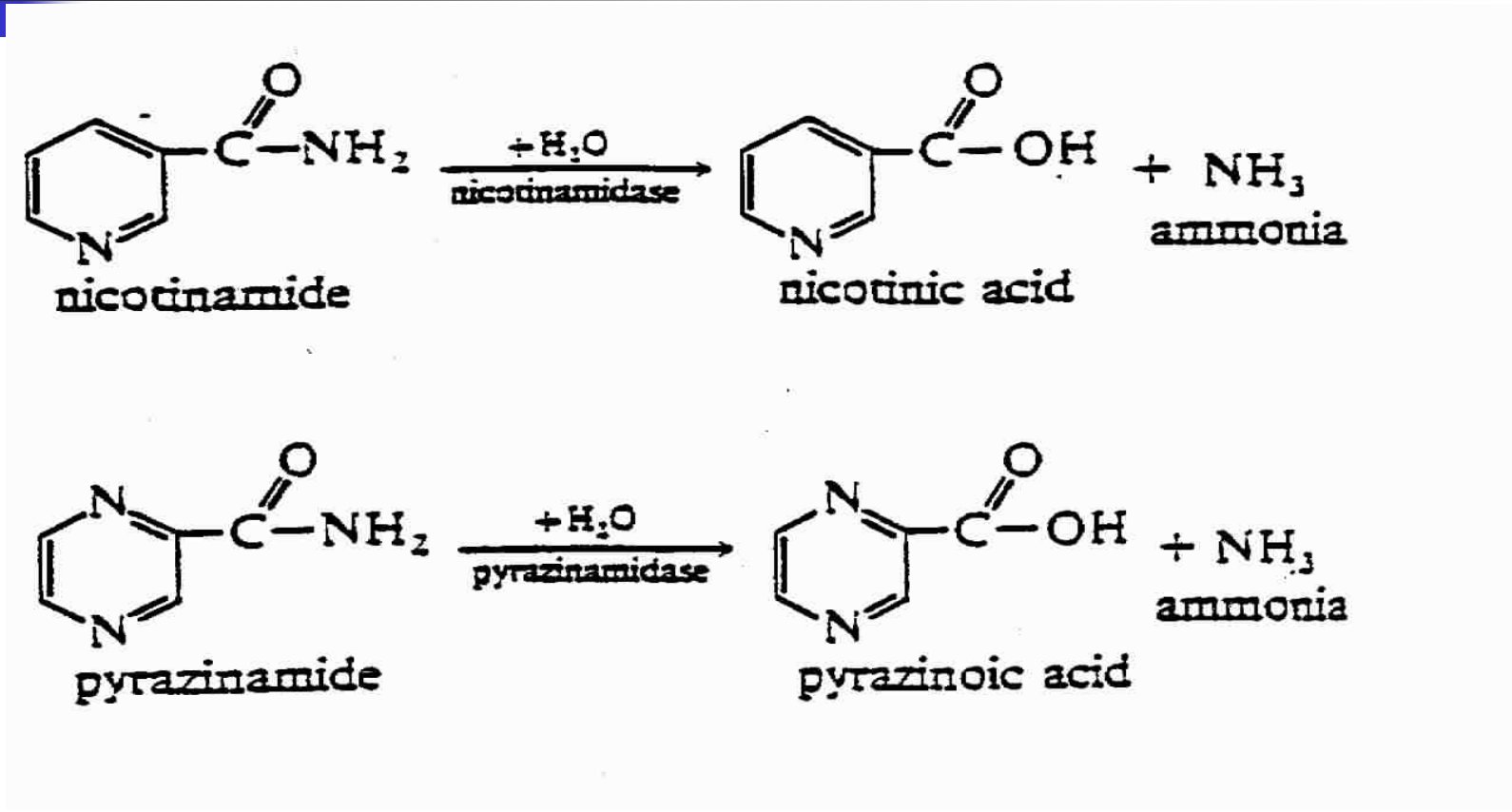
Work on PZA:
(a) acid pH requirement
(b) PZA-resistant TB lose PZase
(c) unique sterilizing activity of PZA in mice
→ Shortening of TB therapy (18-24 months to 6 months)

PZA Has High Sterilizing Activity with INH - Basis for SCC



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

Mechanism of PZA Resistance



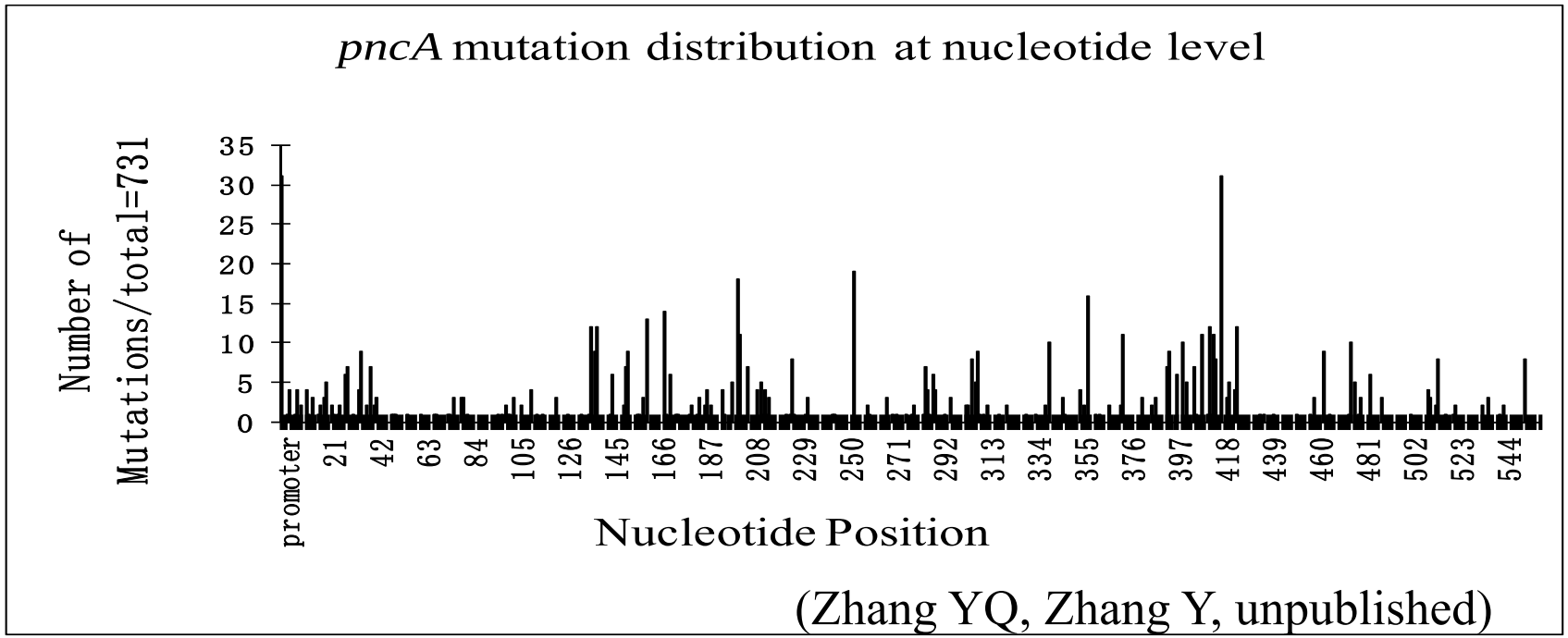
In 1967, Konno and McDermott showed PZA-resistant strains lose pyrazinamidase/nicotinamidase activity

Cloning of TB *pncA* Gene

- Cloned pyrazinamidase gene (*pncA*) and found mutation in *pncA* gene is major mechanism of PZA resistance (Scorpio & Zhang, 1996, Nature Med 2, 662-667)

pncA Mutations: Major Mechanism of PZA Resistance

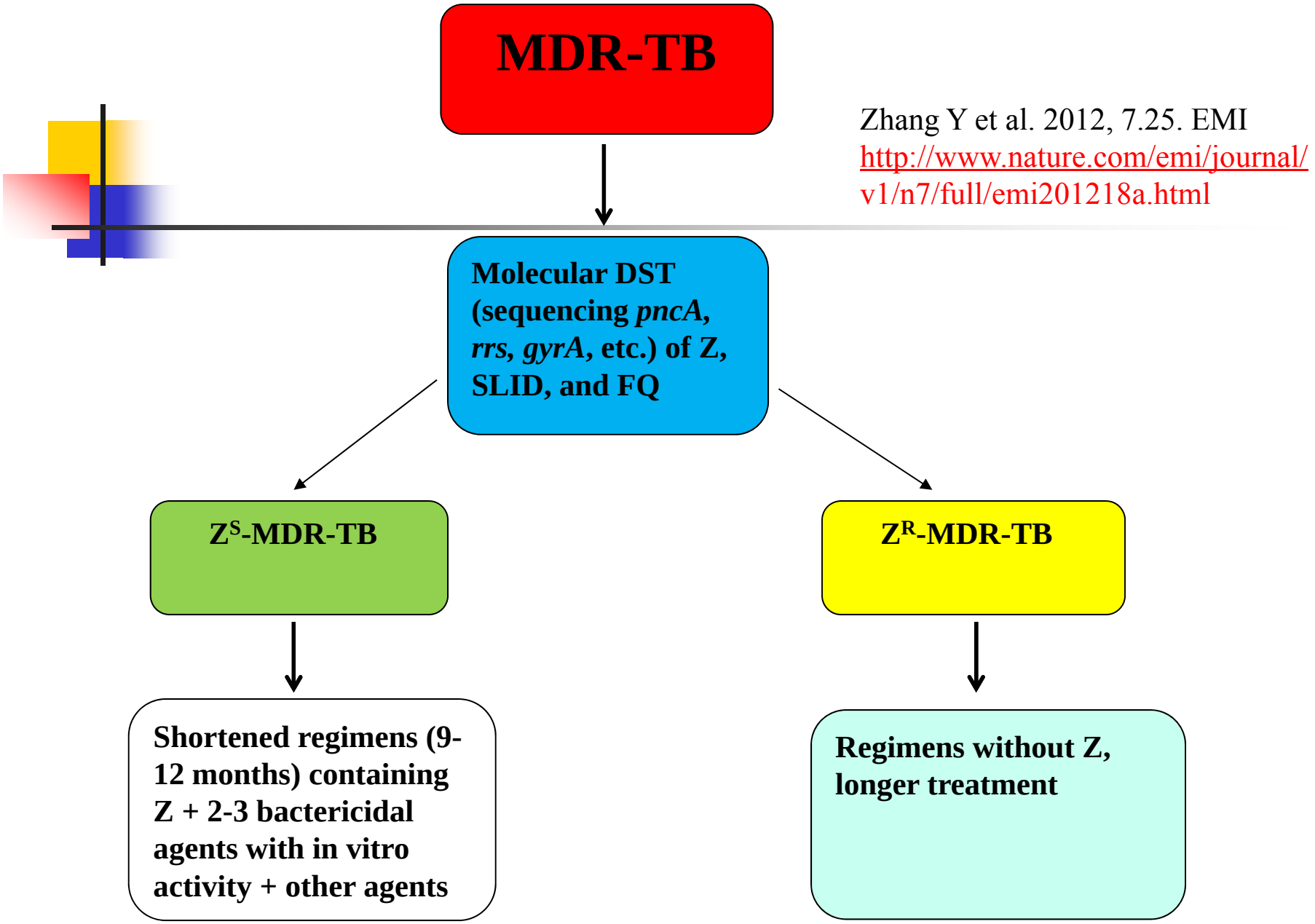
- Mutations in *pncA* gene: major mechanism of PZA resistance, 72-99% (85%), *pncA* mutations are highly diverse
- A few low level PZA-R no *pncA* mutations



(Zhang YQ, Zhang Y, unpublished)

pncA Mutations as a Rapid Test for PZA Resistance

- PZA DST not performed routinely, acid pH, inoculum size, resistance surveys no PZA-R data
- Acid pH inhibits MTB (25-30% acid sensitive)
- BACTEC/MGIT tests at pH 6.0 MIC 100 μg/ml, false resistance →156, 300 μg/ml; takes 2 wks, expensive, not widely used
- *pncA* sequencing (560 bp): rapid PZA DST, good correlation between *pncA* mutations and PZA-R (85%)
- Some mutations found in susceptible strains? Asp12Ala; Ala28Thr; His43Tyr; Thr47Ala; Lys48Thr; Asp49Glu; Thr142Met
- Mayo Clinic in US



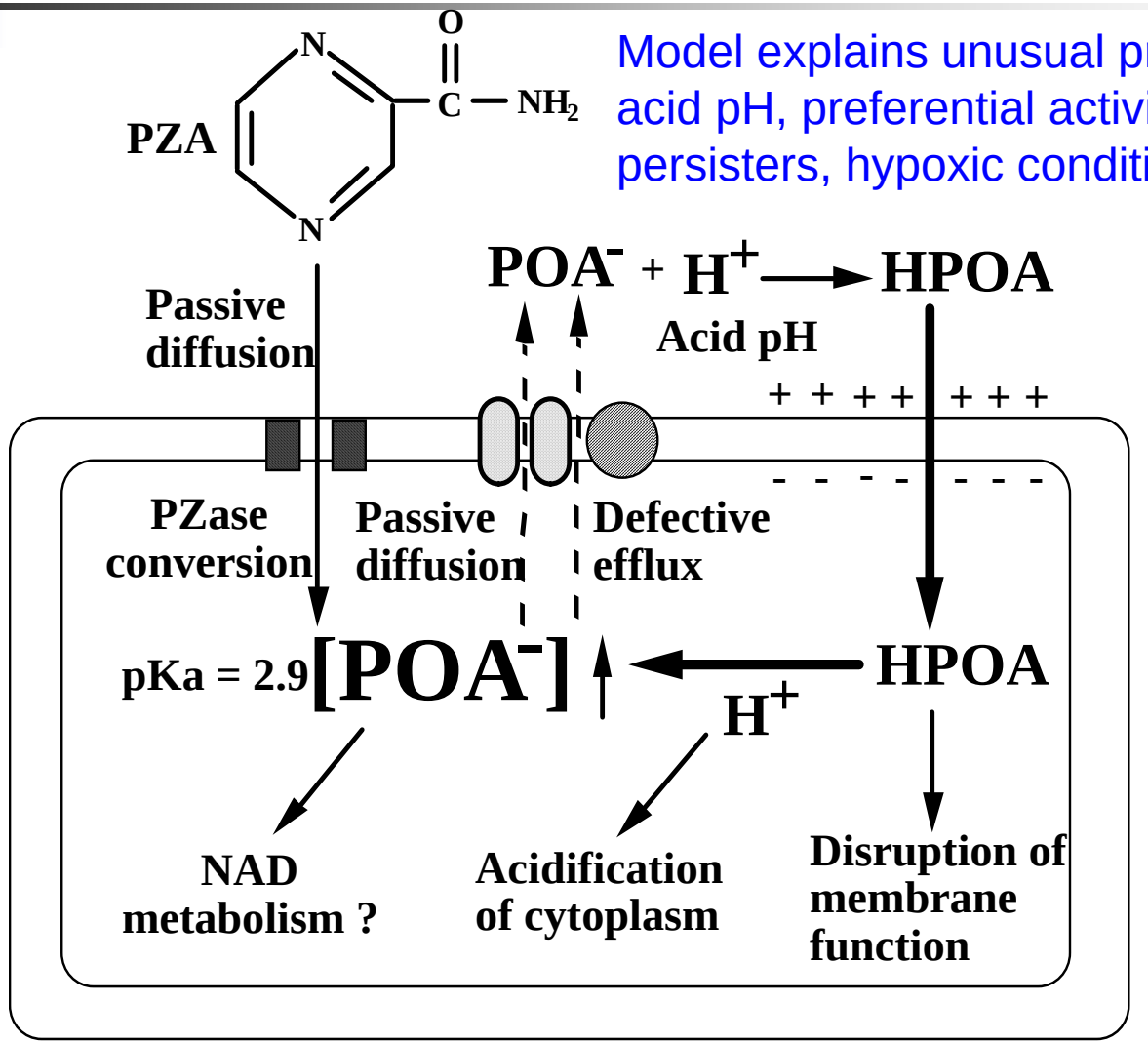
Zhang Y et al. 2012, 7.25. EMI
http://www.nature.com/emi/journal/v1/n7/full/emi201218a.html

How Does PZA Work?

- PZA, prodrug activated by PncA to POA (Scorpio & Zhang 1996)
- Role of acid pH (Zhang et al., 1999)
- Henderson-Hasselbalch equation: relation pH and PZA activity (Zhang et al., 2002)
- PZA kills old, dormant bacilli more effectively (Zhang et al., 2002), kills persisters better under hypoxic/anaerobic (Wade & Zhang, 2004)
- POA disrupts MP, inhibits transport (Zhang et al., 2003)

Mode of Action of PZA

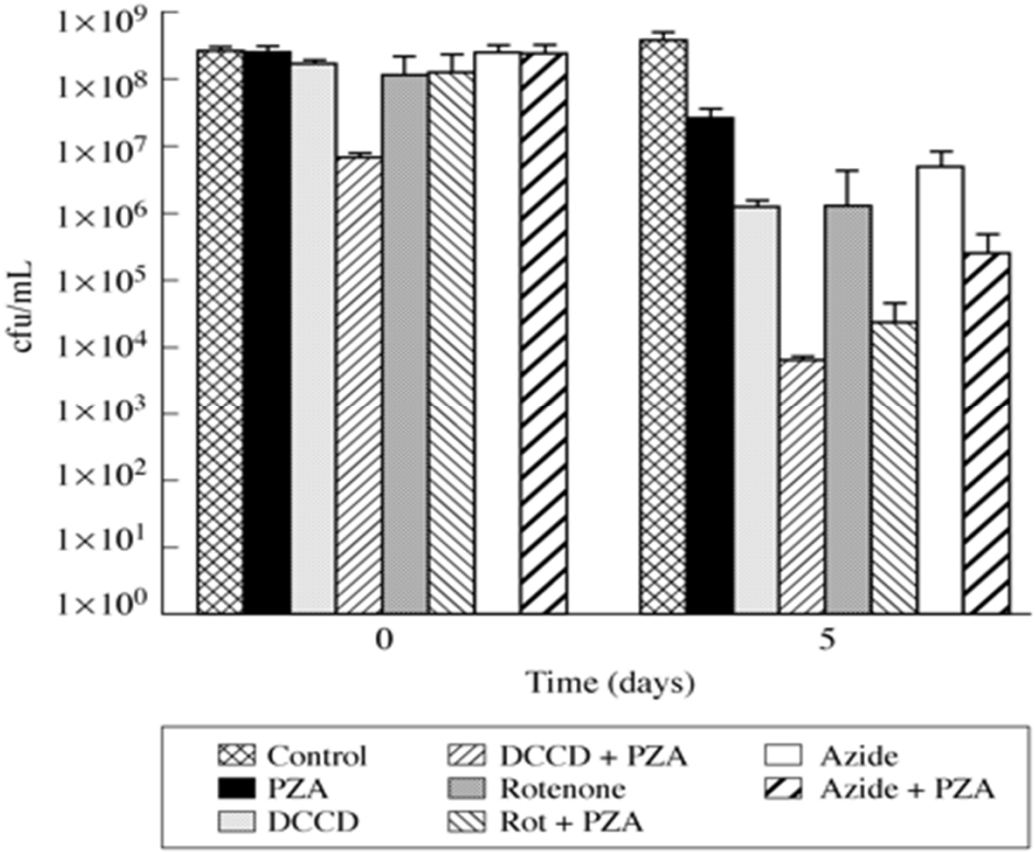
(Zhang et al., J. Antimicrob. Chemother. 2003, 52:790-5)



Model explains unusual properties of PZA: acid pH, preferential activity for non-replicating persisters, hypoxic conditions, predict…

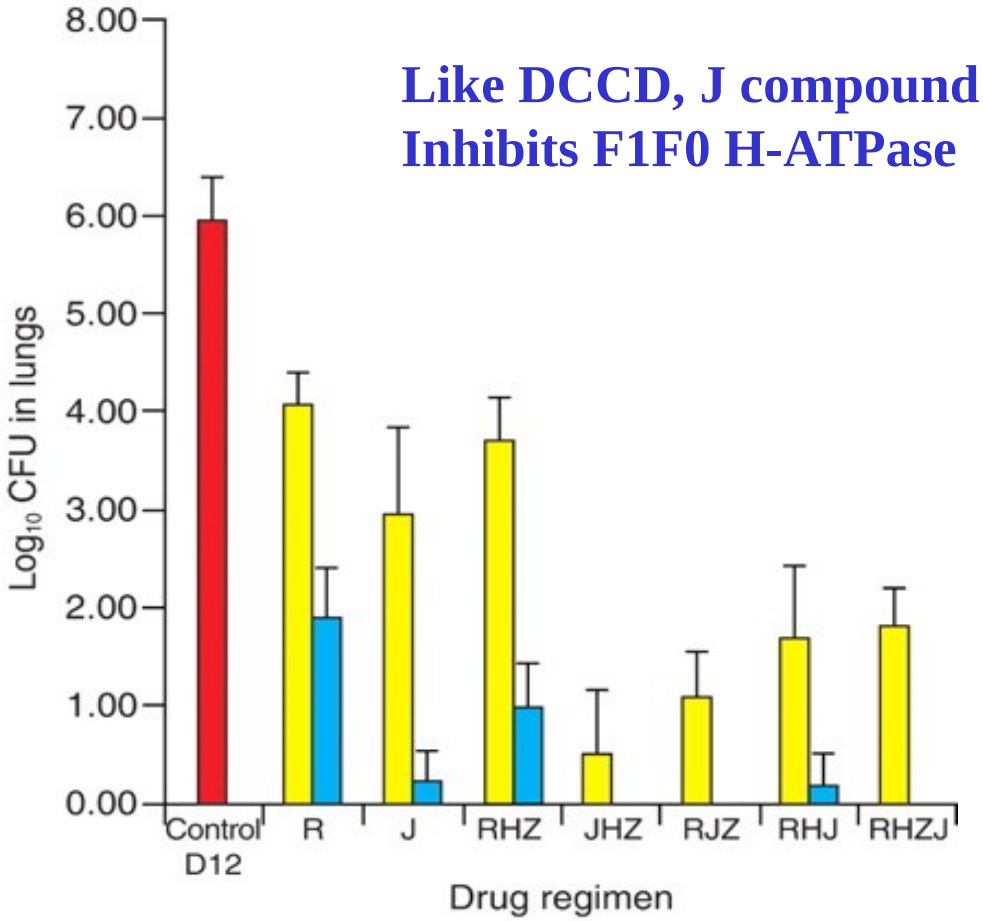
PZA Activity Enhanced by Energy Inhibitors

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

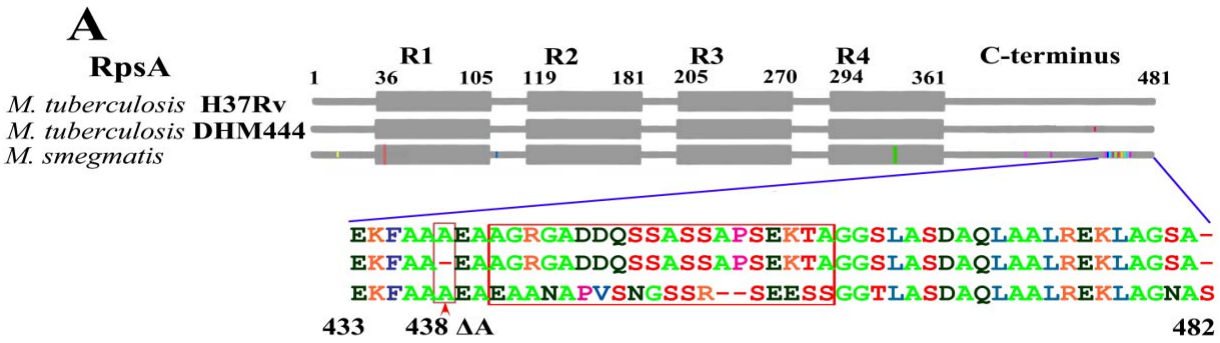
A New Target of PZA: RpsA

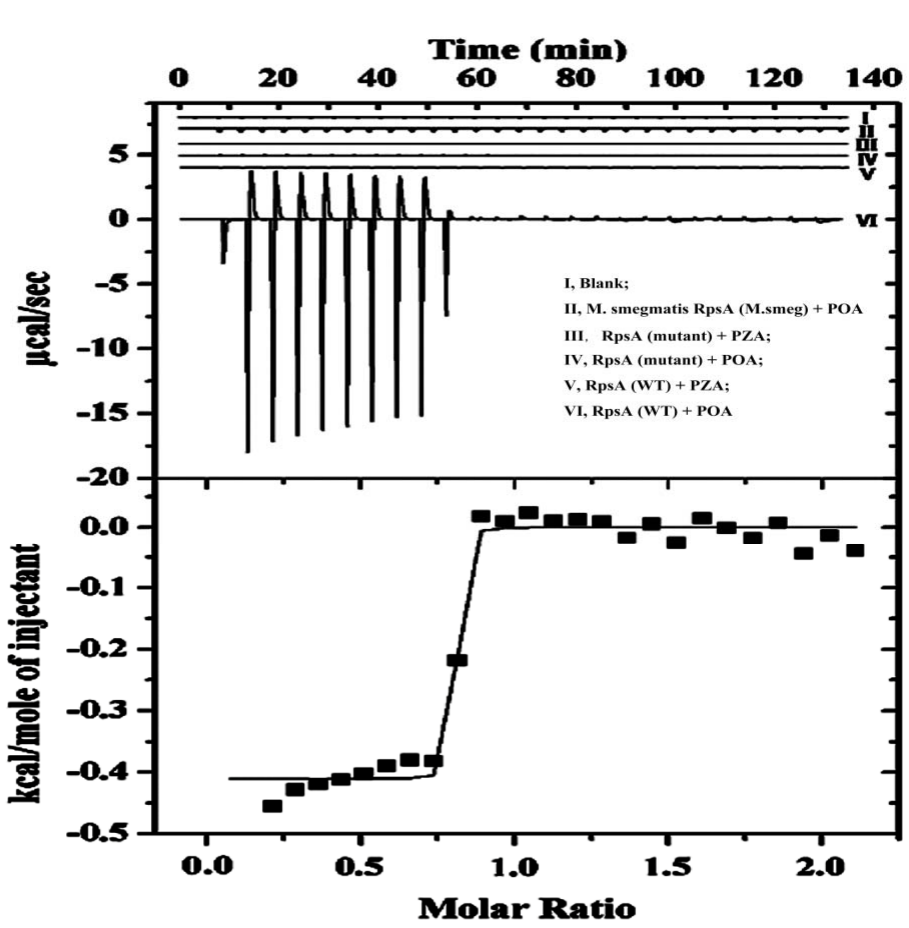
(Shi et al. Science, 2011, 333: 1630-2)

A new target of PZA: POA binds RpsA (S1 protein)

RpsA overexpression conferred 5-fold PZA resistance from 100 to 500 μg/ml

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





K=(7.53±2.21)×10^6 M^{-1}

RpsA mutations:

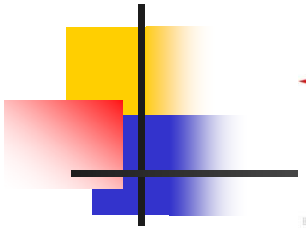
438 ΔA

T5S and D123A

V262M

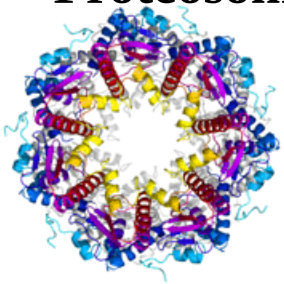
(Shi et al. Science, 2011, 333: 1630-2)

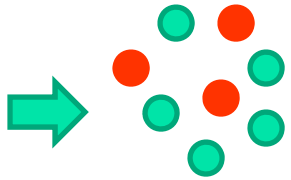
Trans-translation



stress

Proteosome

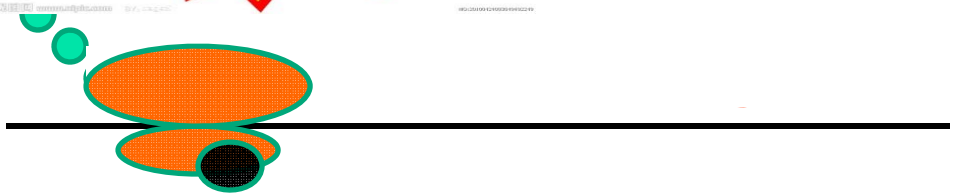




Protein degradation

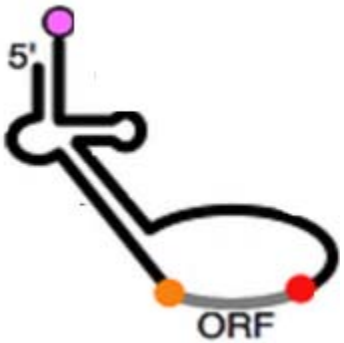
Ribosome recycling

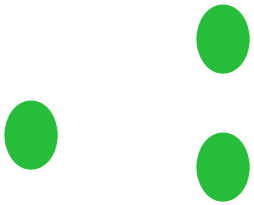
mRNA

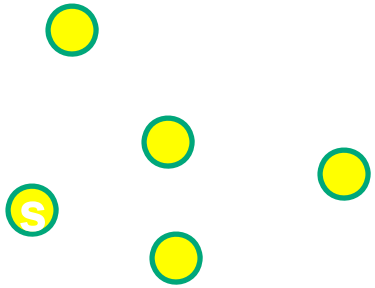


RpsA

mRNA degradation







EF-Tu

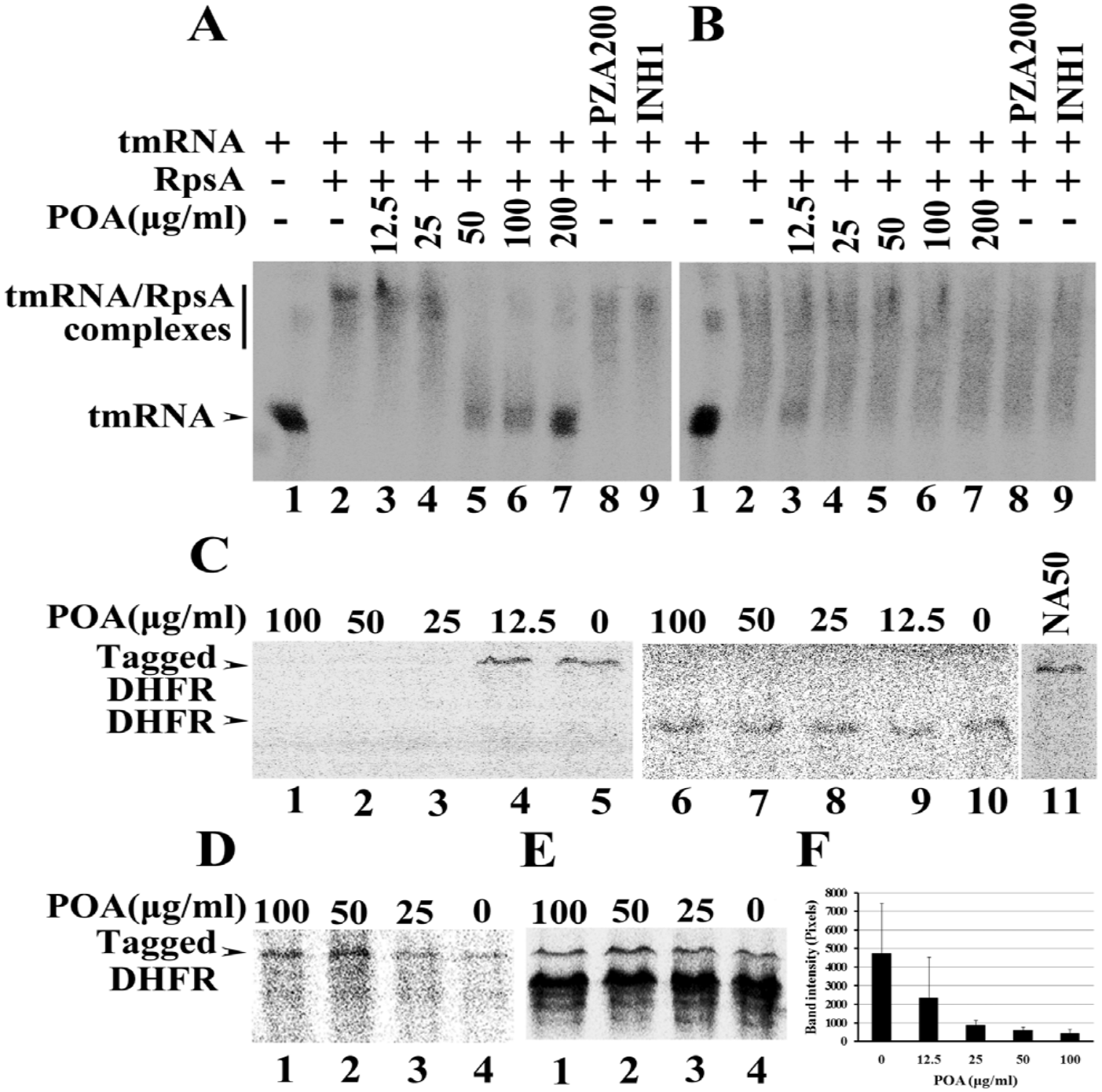
tmRNA

SmpB

RpsA (S1) and Trans-translation

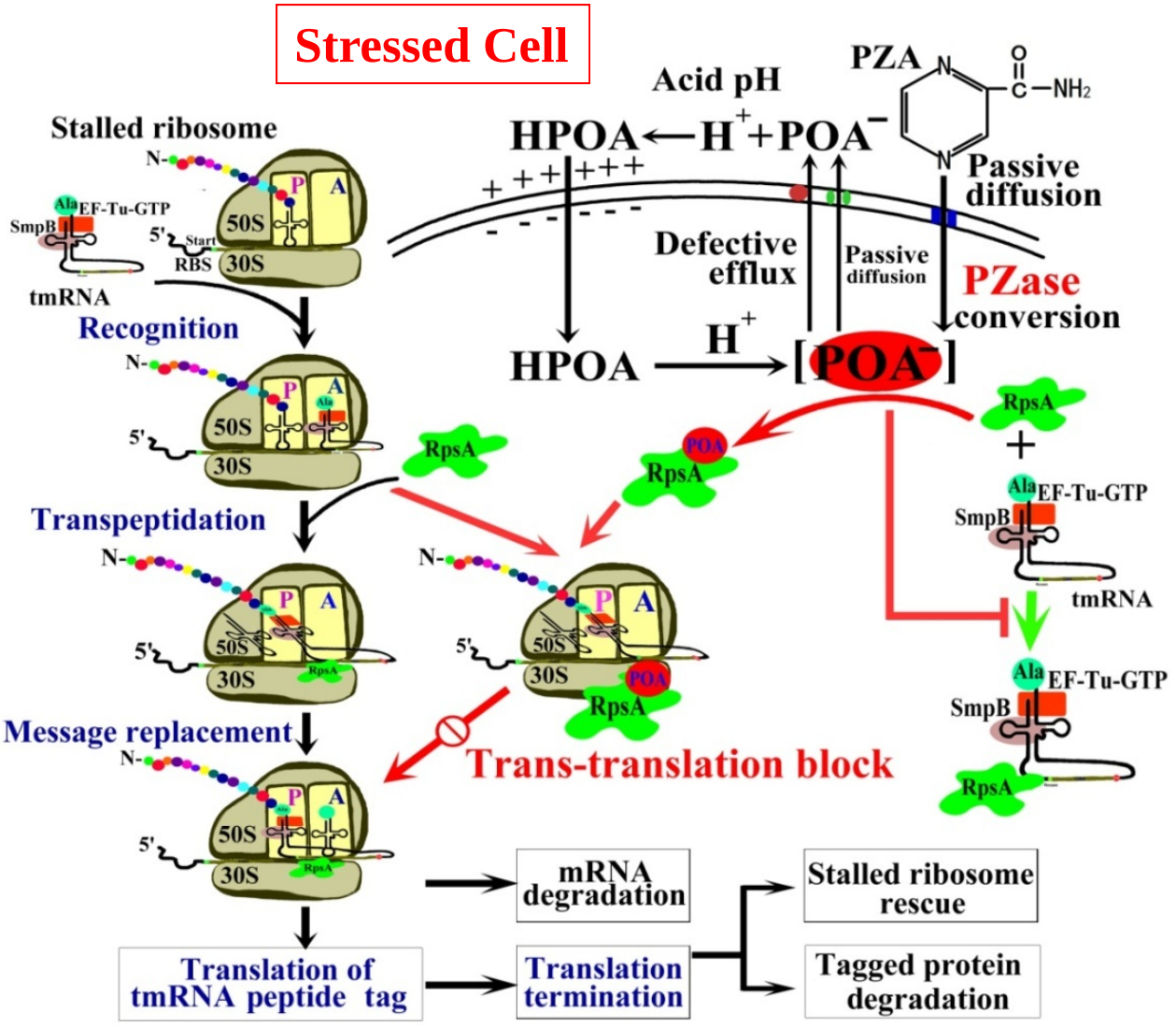
Trans-translation, ubiquitous, is dispensable during growth, but critical under stress, remove stalled ribosomes, damaged mRNA and toxic proteins → stress survival (L-form) and virulence (*Y. pestis*, *H. pylori*, *S. typhi*)

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


A
B
tmRNA
RpsA
POA(µg/ml)
PZA200
INH1
tmRNA/RpsA complexes
tmRNA
1 2 3 4 5 6 7 8 9
C
NA50
Tagged DHFR
DHFR
1 2 3 4 5 6 7 8 9 10 11
D
E
F
Band intensity (Pixels)
POA (µg/ml)

PZA Interferes with Multiple Targets

(Shi et al. Science, 2011, 333: 1630-2)



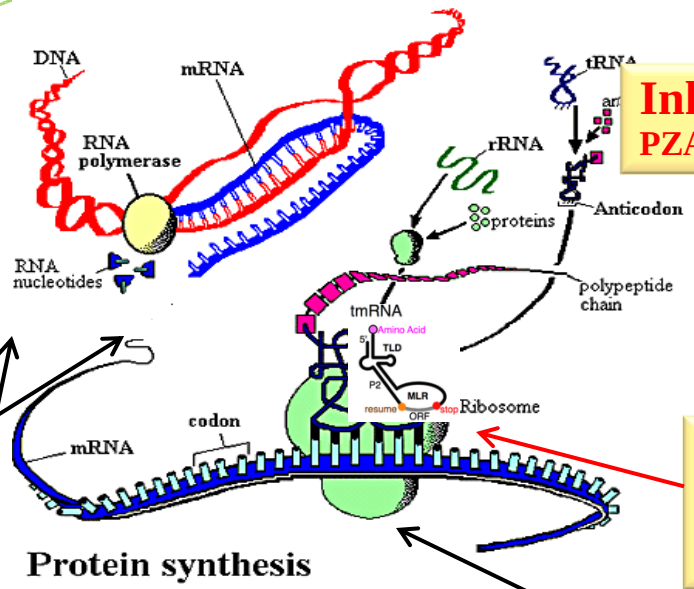
Mechanisms of Action of Antibiotics

Inhibition of cell wall synthesis
- Beta-lactams
- Glycopeptides

Disruption of membrane permeability
- Polymyxin B
- Daptomycin

Anti-metabolite
- Sulfa drugs

THFA → DHFA → PABA



Inhibition of Energy Production
PZA, TMC207

ATP → ADP+Pi

Inhibition of trans-translation (PZA), a new persister target, for a new generation of antibiotics

Inhibition of nucleic acid synthesis
- Quinolones
- Rifampin

Inhibition of protein synthesis
- Aminoglycosides
- Tetracycline
- Macrolides

PZA and New TB Drug Candidates – Indispensable, Synergy

<u>Drug candidates under clinical development:</u>

-Rifapentine: Phase II

-Linezolid: Phase I and II

-Moxifloxacin/gatifloxacin, Phase II, III

-Diarylquinoline (TMC207): Phase II (MDR-TB, DS-TB)

-Nitroimidazoles: PA-824 and OPC-67683, Phase II trials

-Ethambutol analog, SQ-109, Phase II

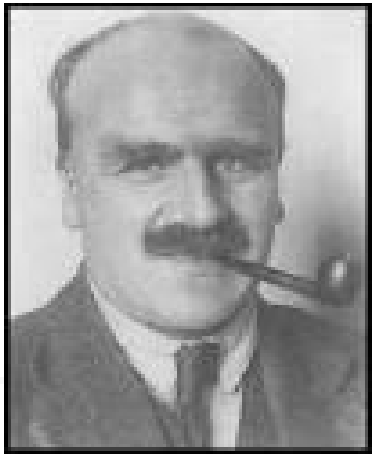
Limitation of current drug discovery: None can replace PZA

CPTR: Build new regimens: PZA + TMC207 or PA-824 +…

Future Studies

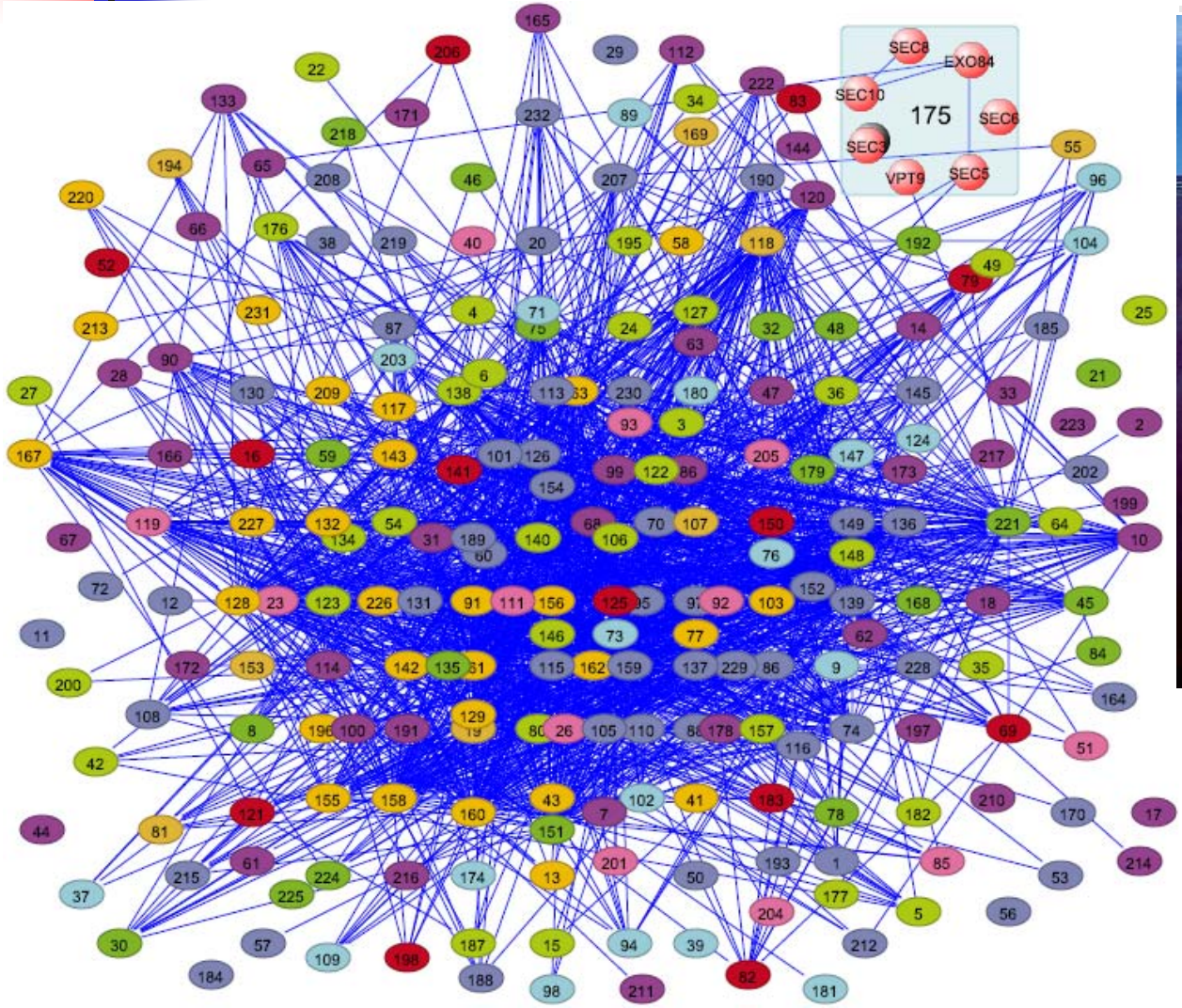
- Mechanisms of Action Studies: new PZA targets and mutations, crystal structures, role of targets
- Drug Screens → inhibitors of trans-translation
- Shorten TB Therapy: Z-combinations
- Rapid Detection of PZA Resistance: *pncA* sequencing
- Shorten MDR-TB Treatment based on PZA DST:
  Z^S-MDR vs Z^R-MDR

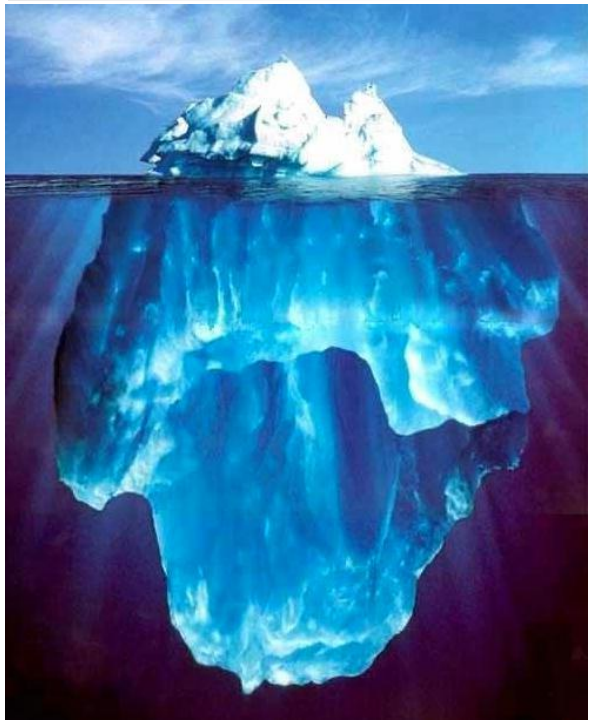
Four Stages of Scientific Acceptance – J.B.S. Haldane (1892 - 1964)



1. This is worthless nonsense - PZA inhibits trans-translation
2. This is an interesting, but perverse, point of view - PZA kills persisters (Yin) not growing bacilli (Yang)
3. This is true, but quite unimportant - PZA disrupts MP
4. I always said so - *pncA* mutations cause PZA resistance

PZA MOA: Complex



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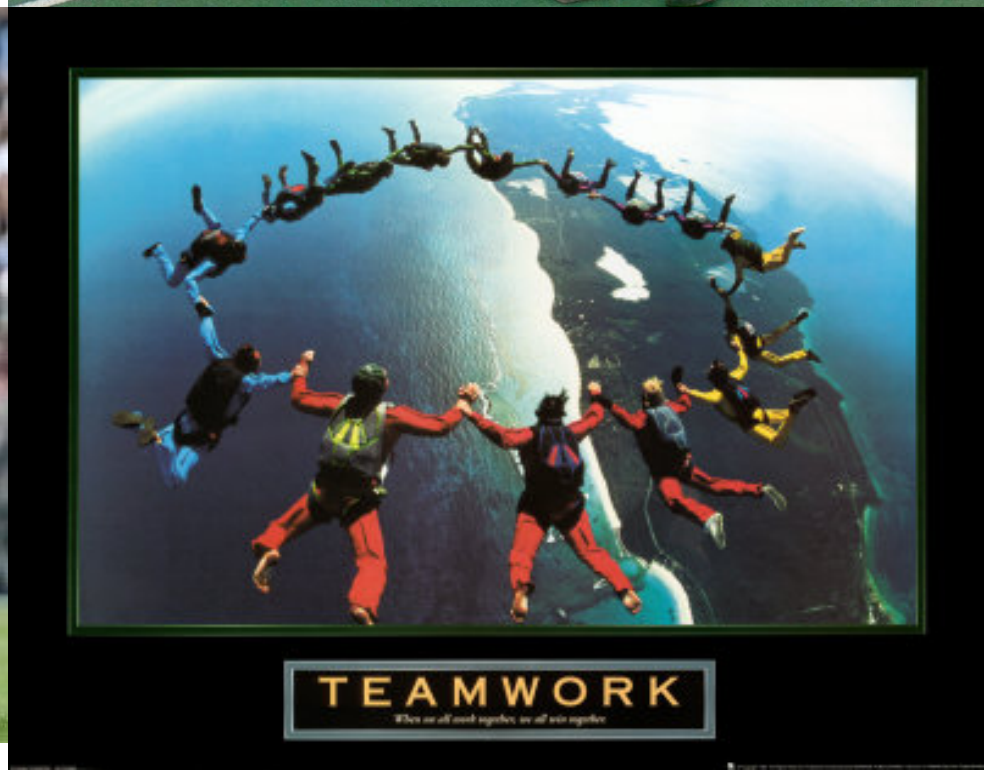
TEAMWORK

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Thank You!
Libaiwa