



12th Annual Conference IUATLD-NAR

- Dr. Christopher Sasseti 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.

FIGHT TUBERCULOSIS

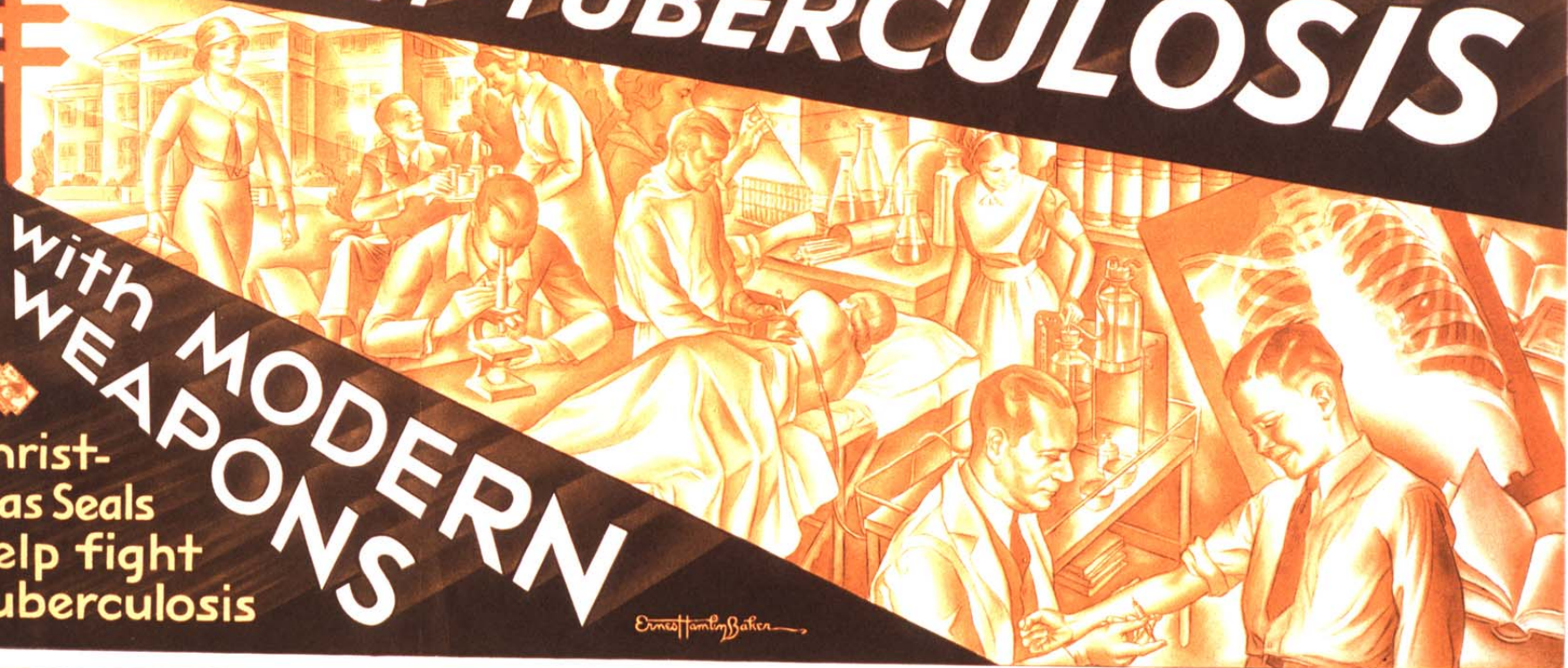


with MODERN WEAPONS

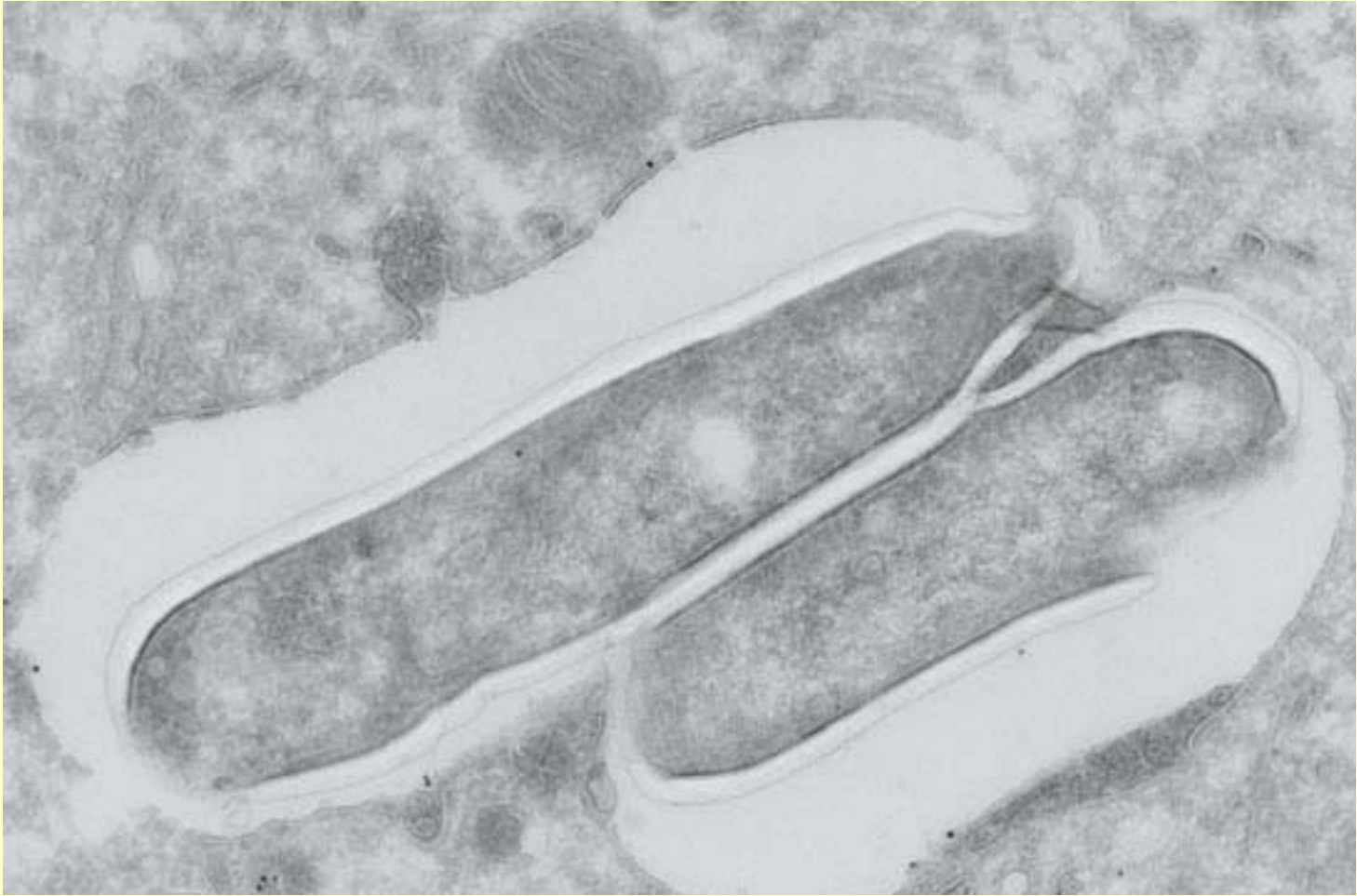
Christmas Seals help fight Tuberculosis

Ernest Tomby Baker

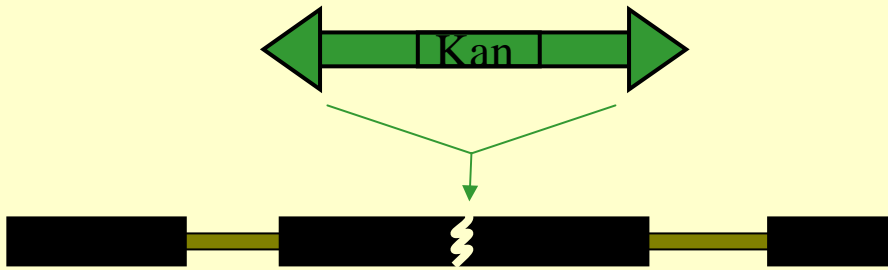
© 1945 A.P.C.



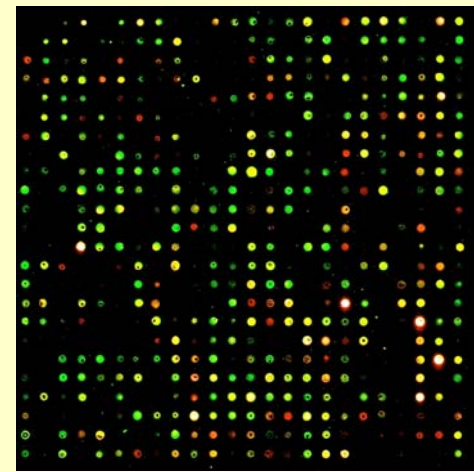
Mycobacterium tuberculosis



van der Wel et al. Cell, 129:1287-98 (2007)



Transposon mutagenesis

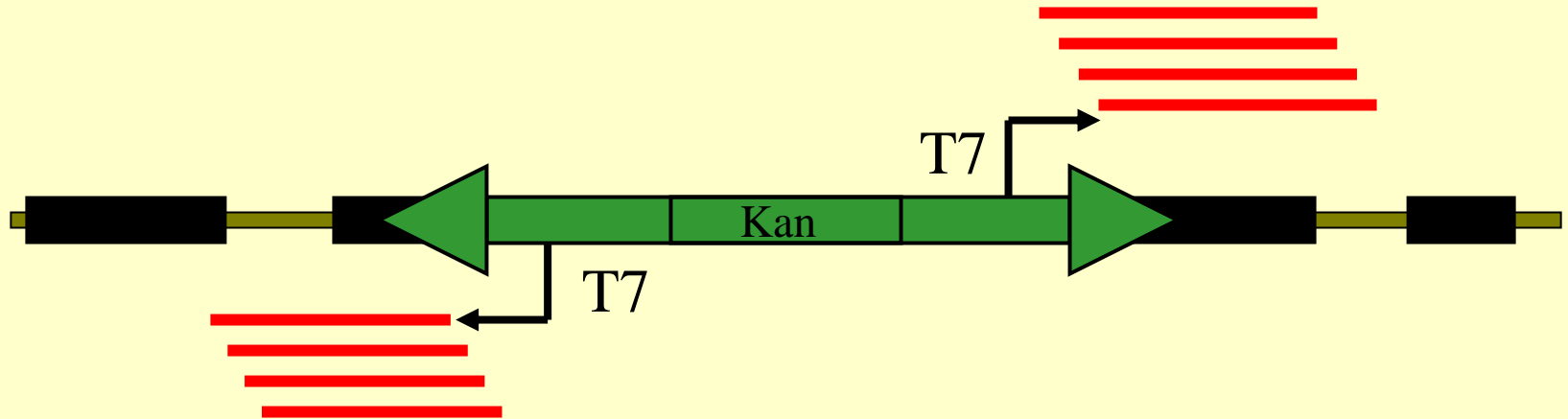


Microarray mapping of
insertion sites

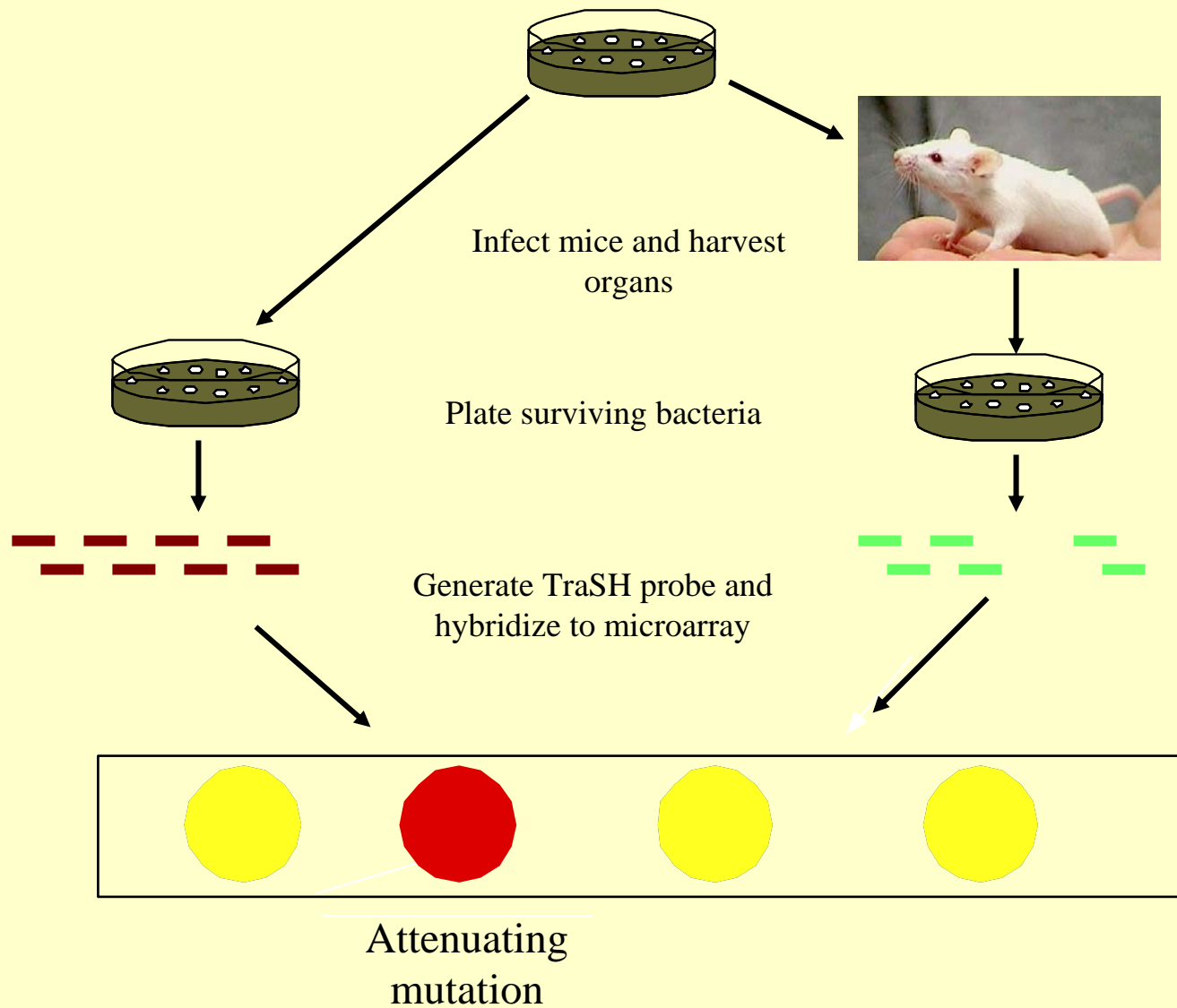
Transposon site hybridization
(TraSH)

Identify mutants that are unable to survive
under a particular growth condition
= Genes that are required for growth

T7 transposon

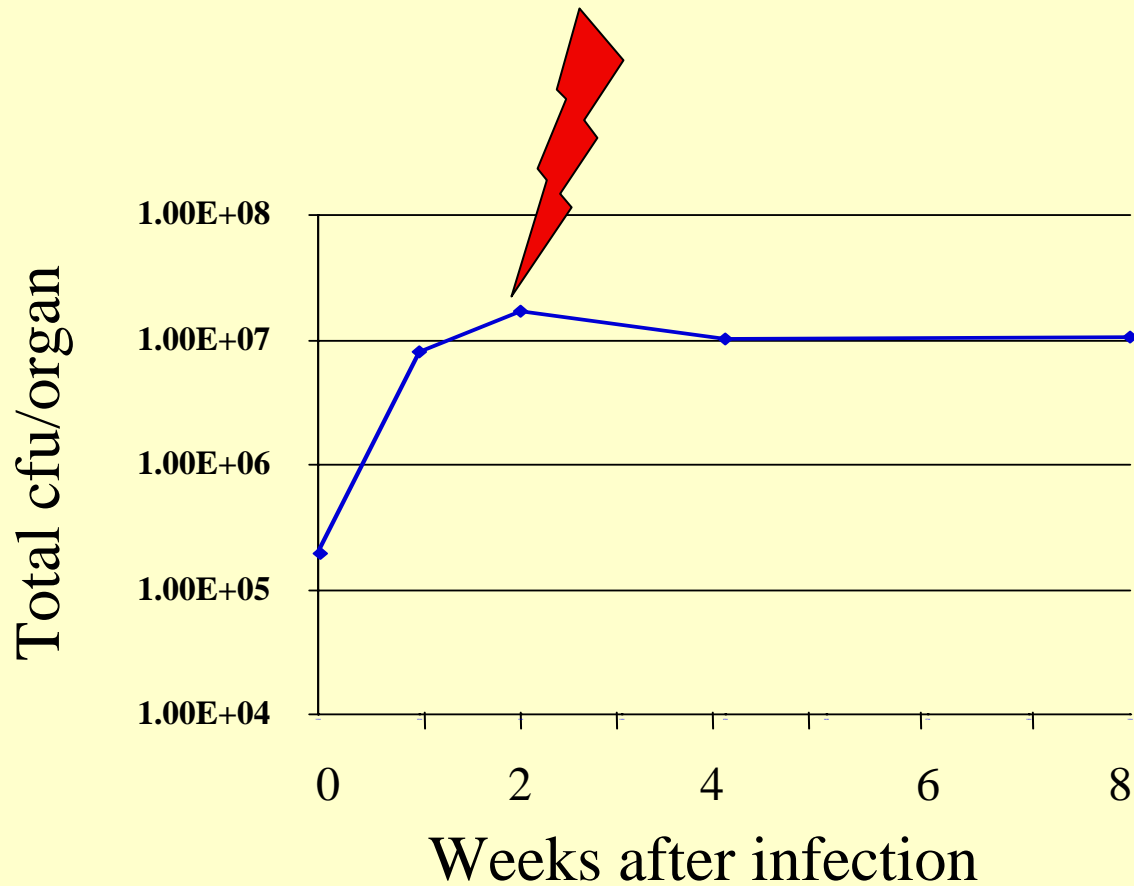


Genes required for murine infection

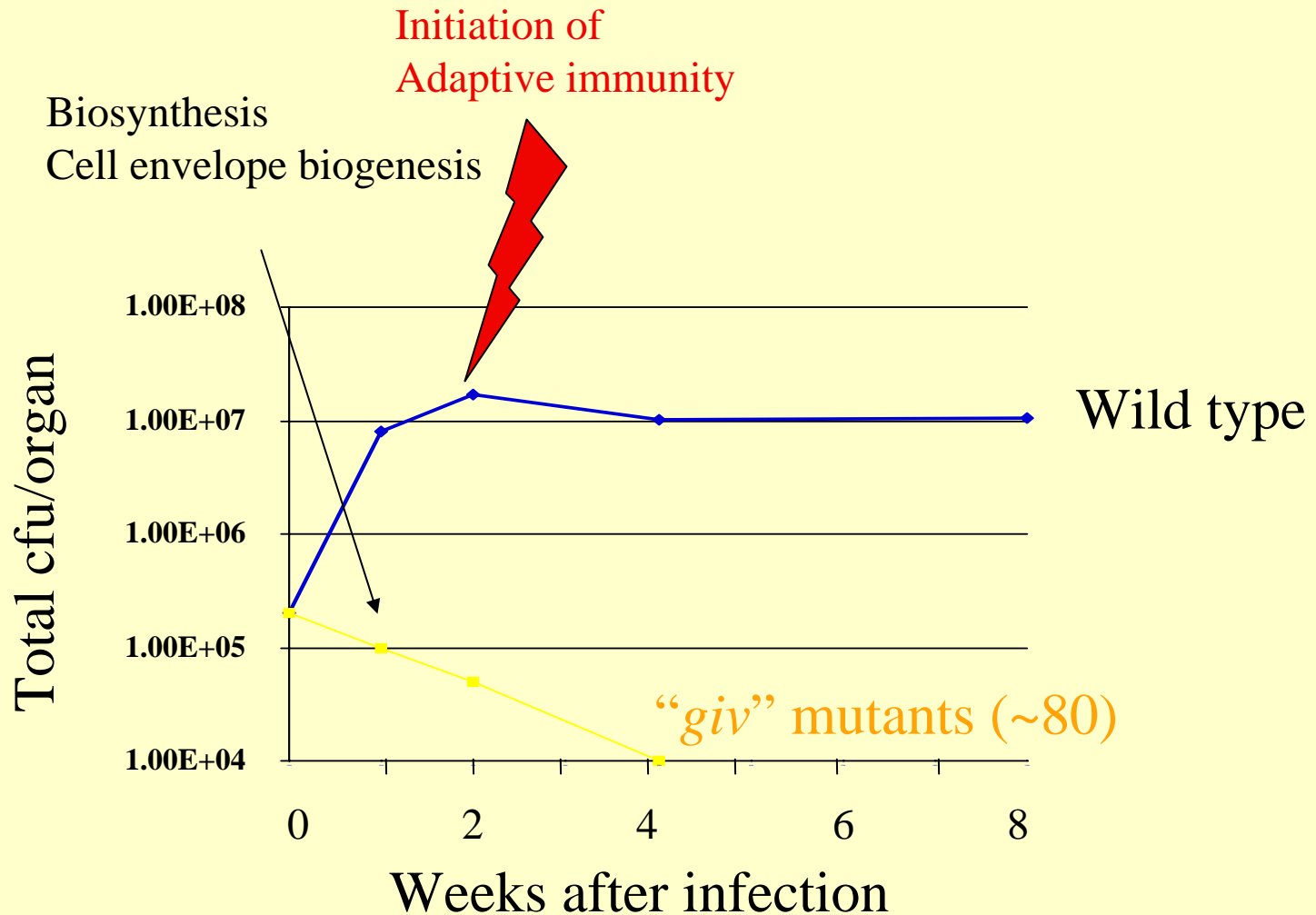


The host environment changes over time

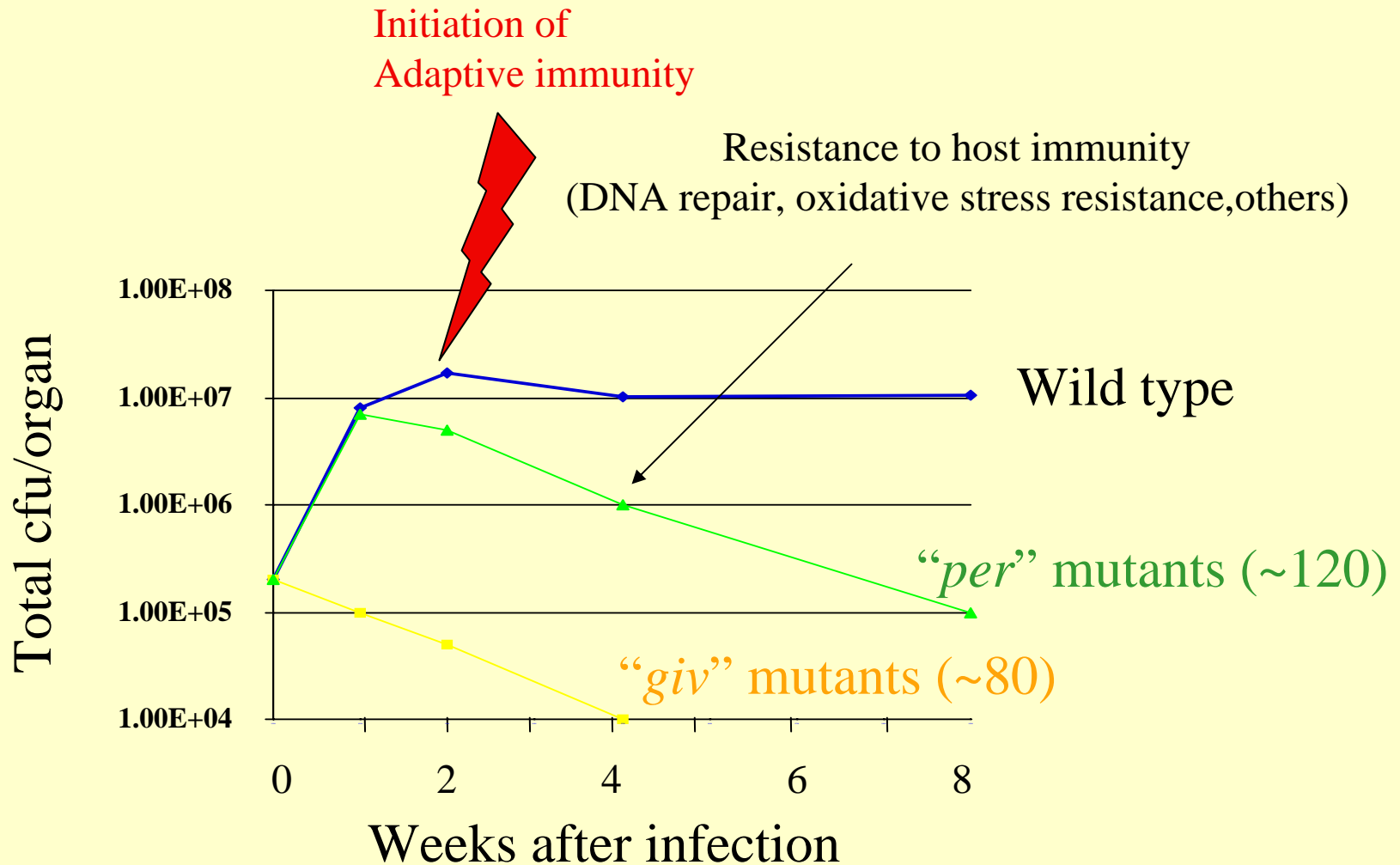
Initiation of
Adaptive immunity



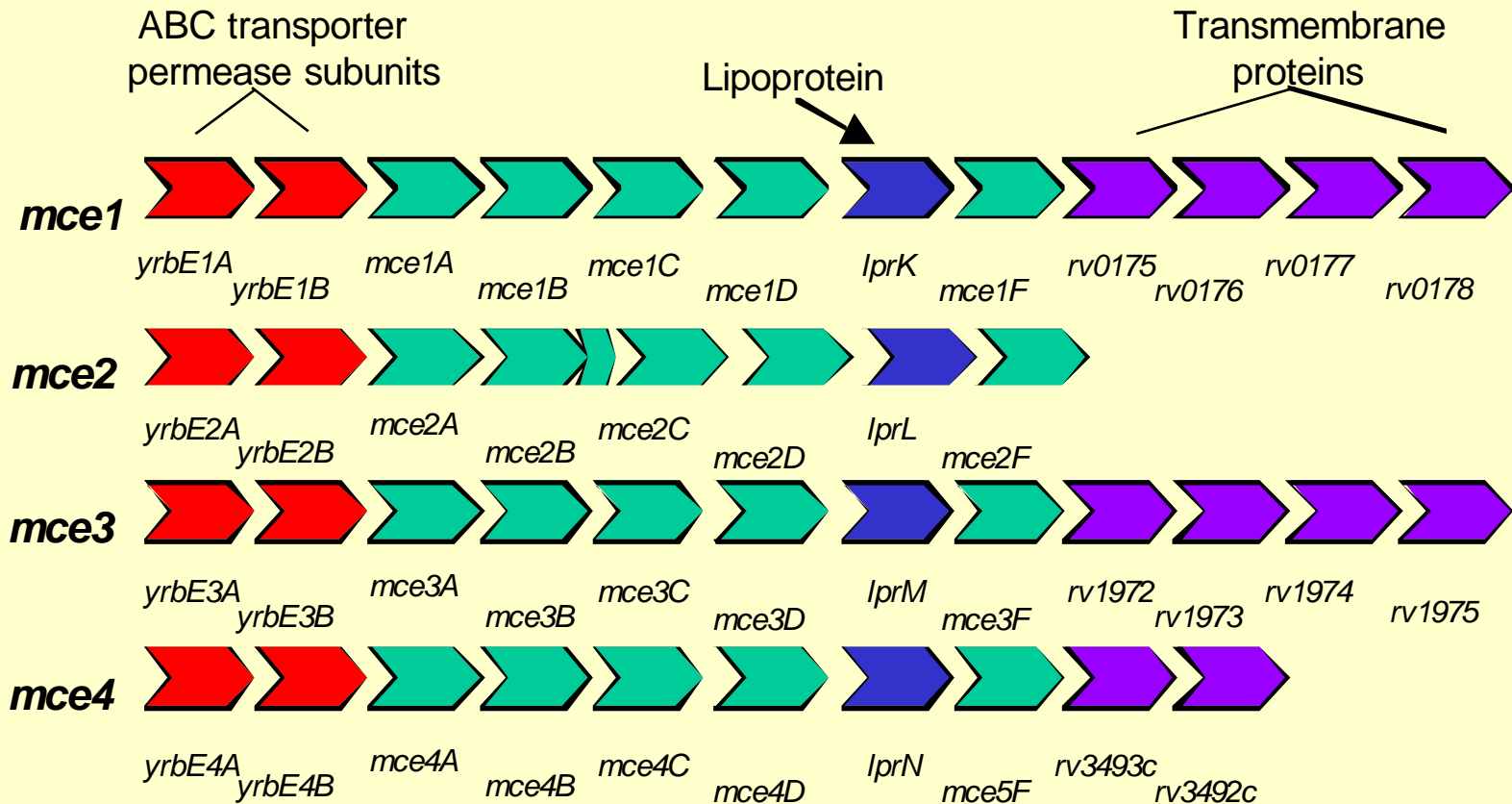
The host environment changes over time



The host environment changes over time

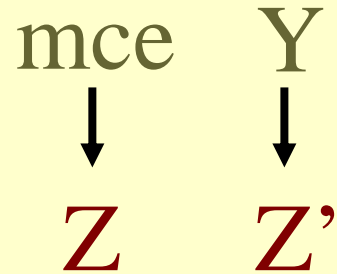


Mce loci of *M. tuberculosis*

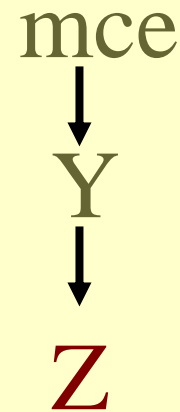


Pathway mapping using genetic interactions

Independent pathways

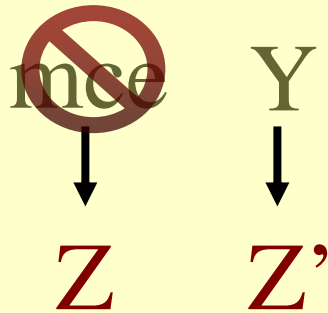


Dependent pathway

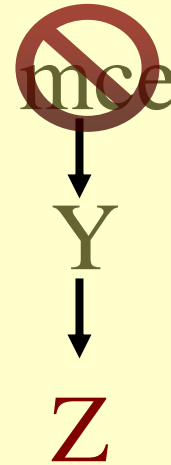


Pathway mapping using genetic interactions

Independent pathways

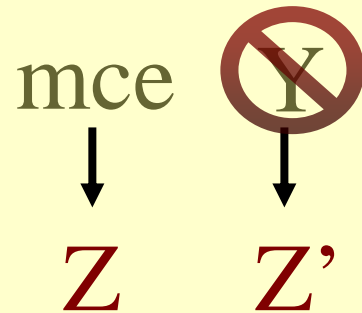


Dependent pathway

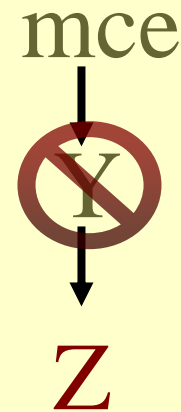


Pathway mapping using genetic interactions

Independent pathways

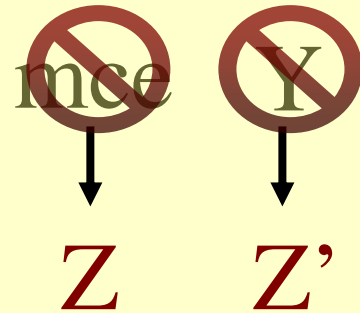


Dependent pathway

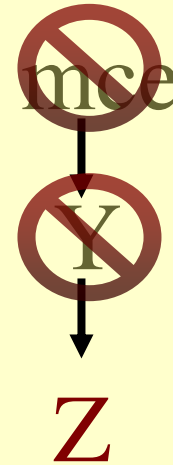


Pathway mapping using genetic interactions

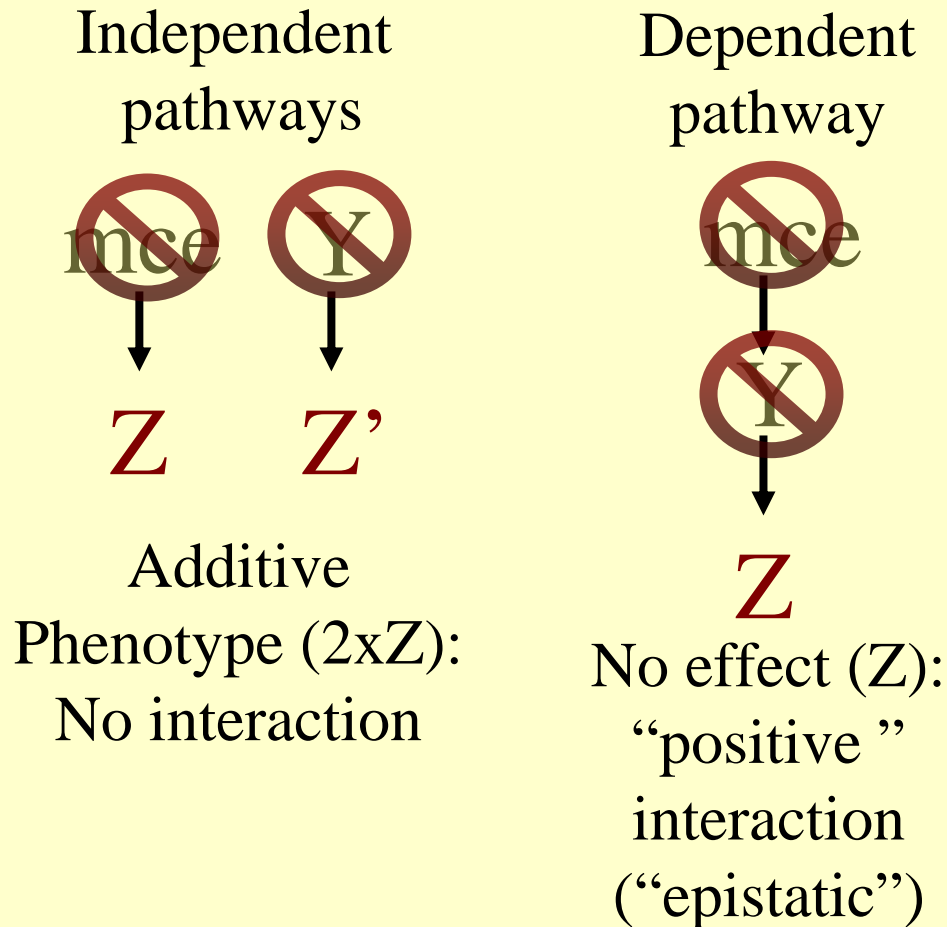
Independent pathways



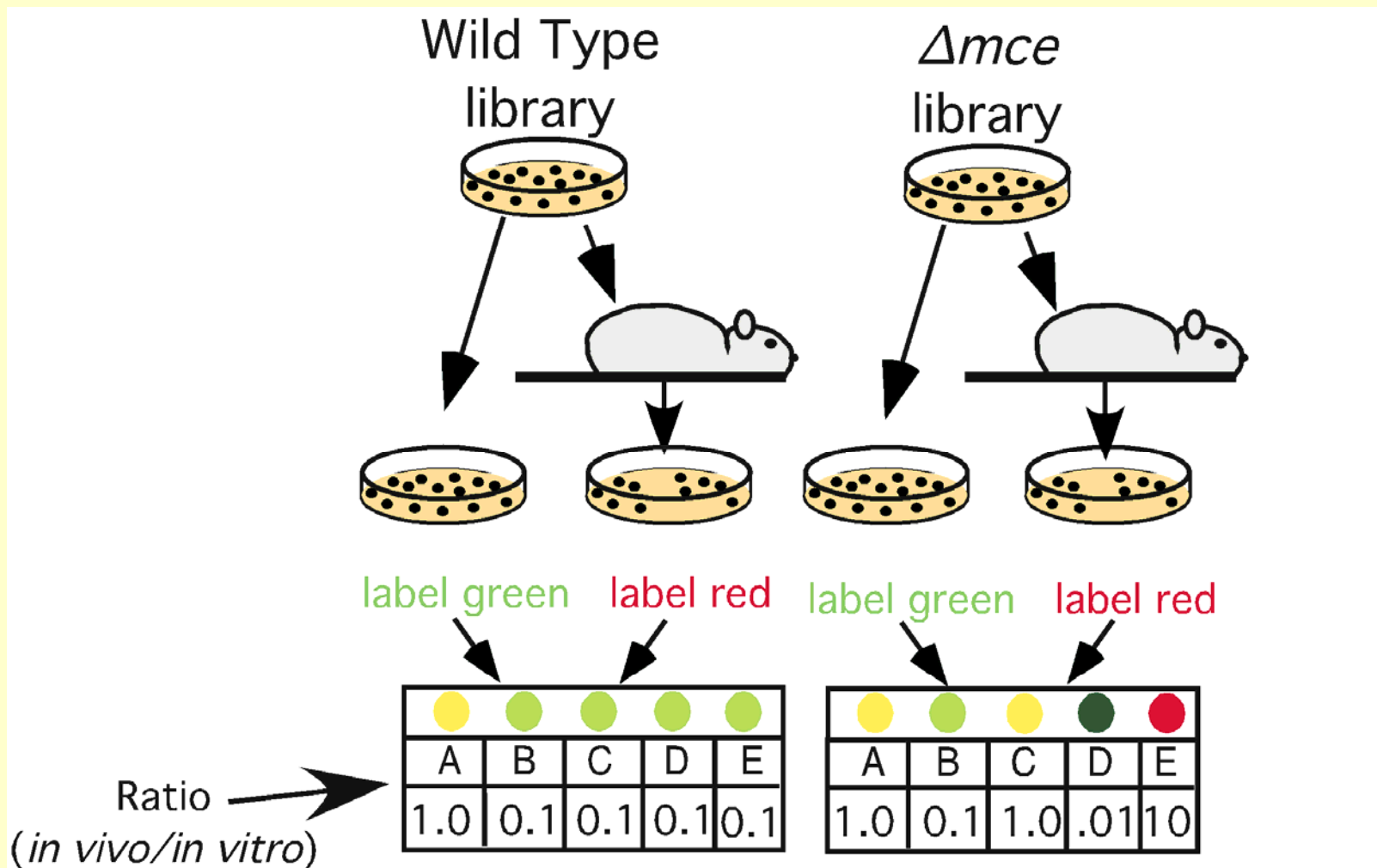
Dependent pathway



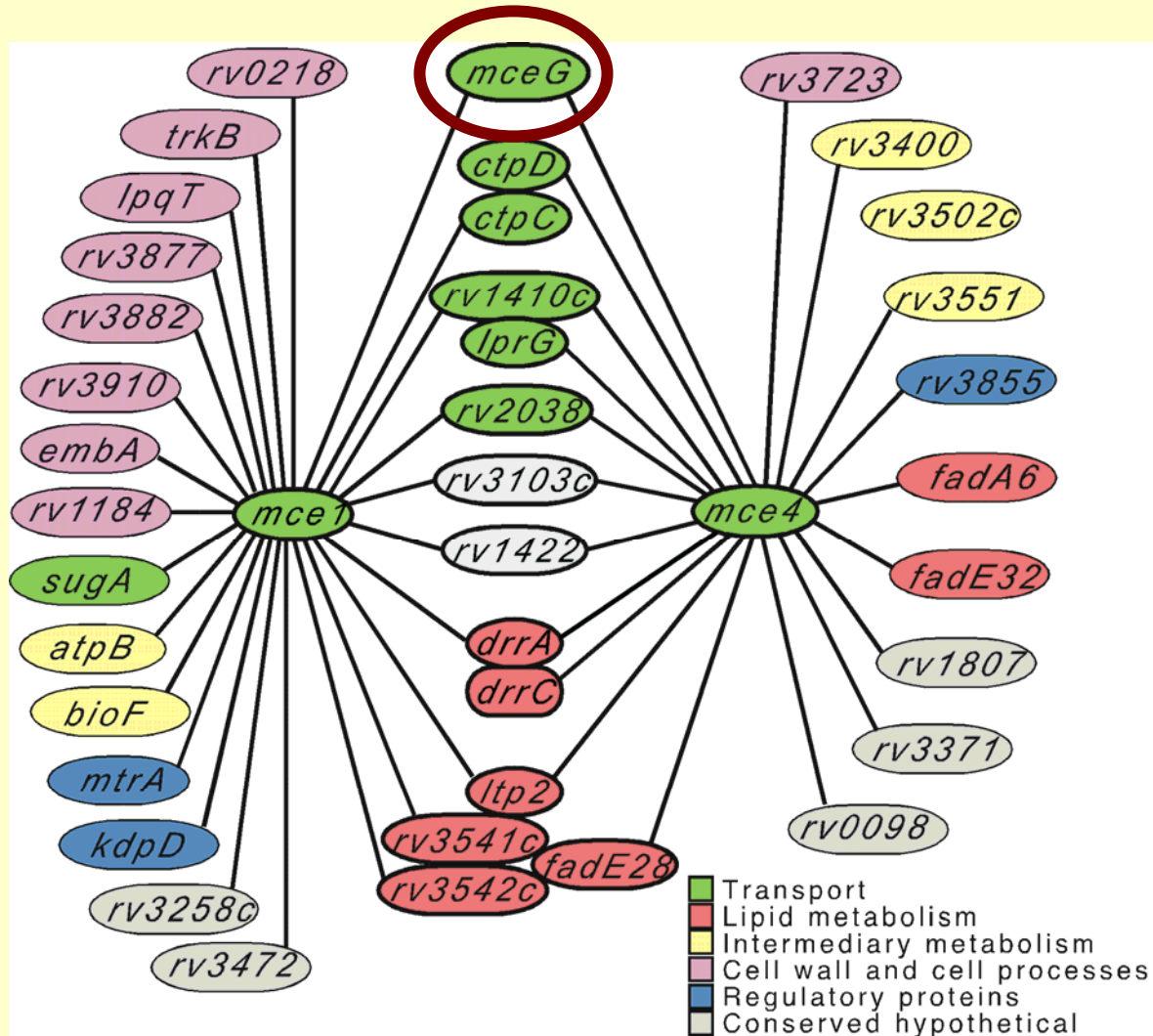
Pathway mapping using genetic interactions



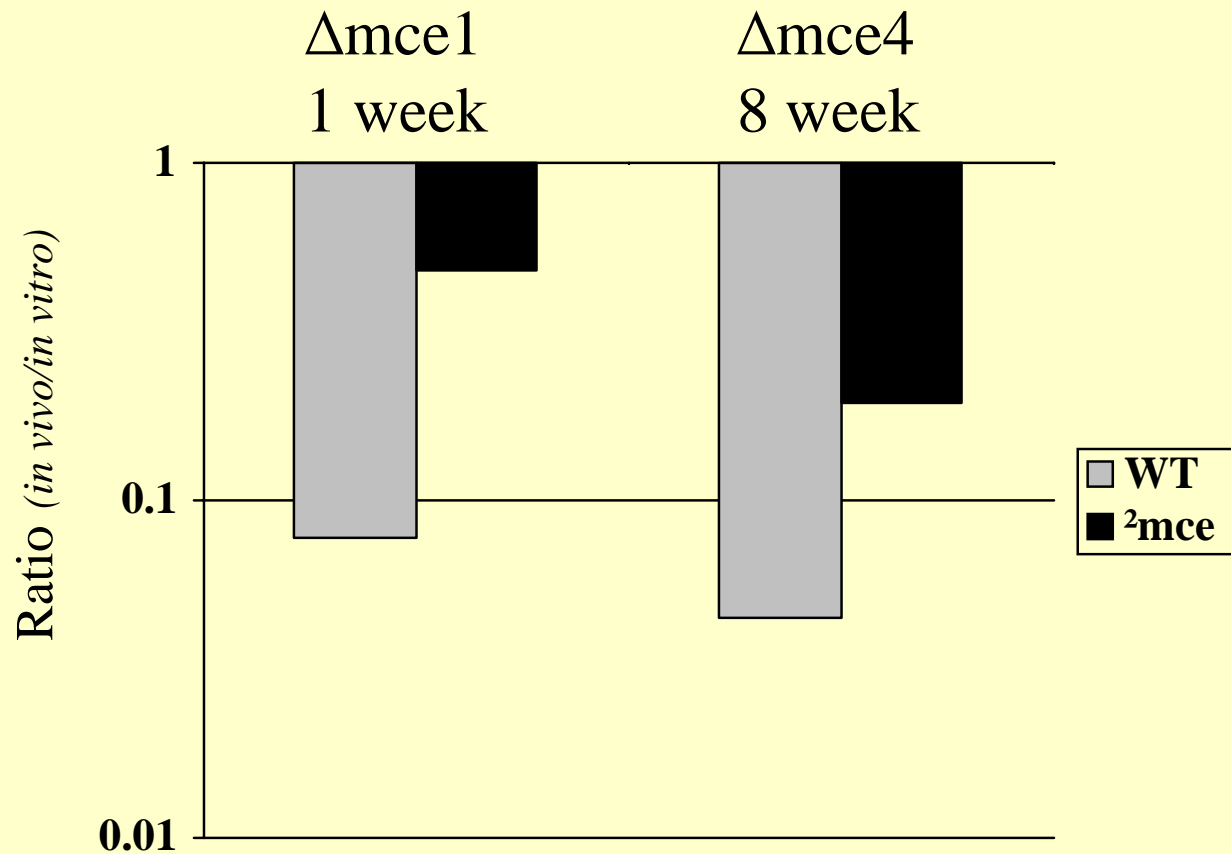
Strategy to identify genes that interact with the *mce* loci



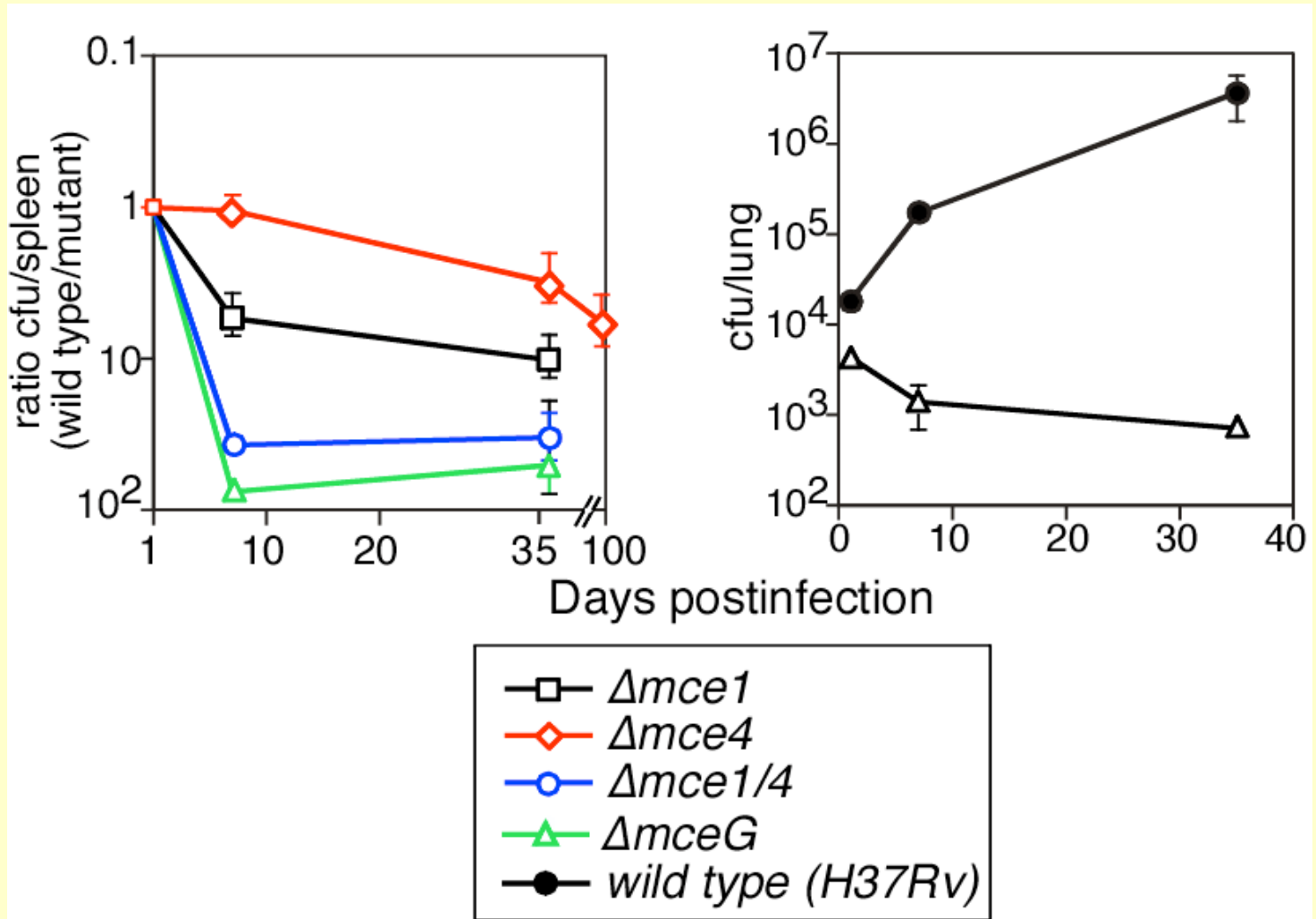
Mce interaction map



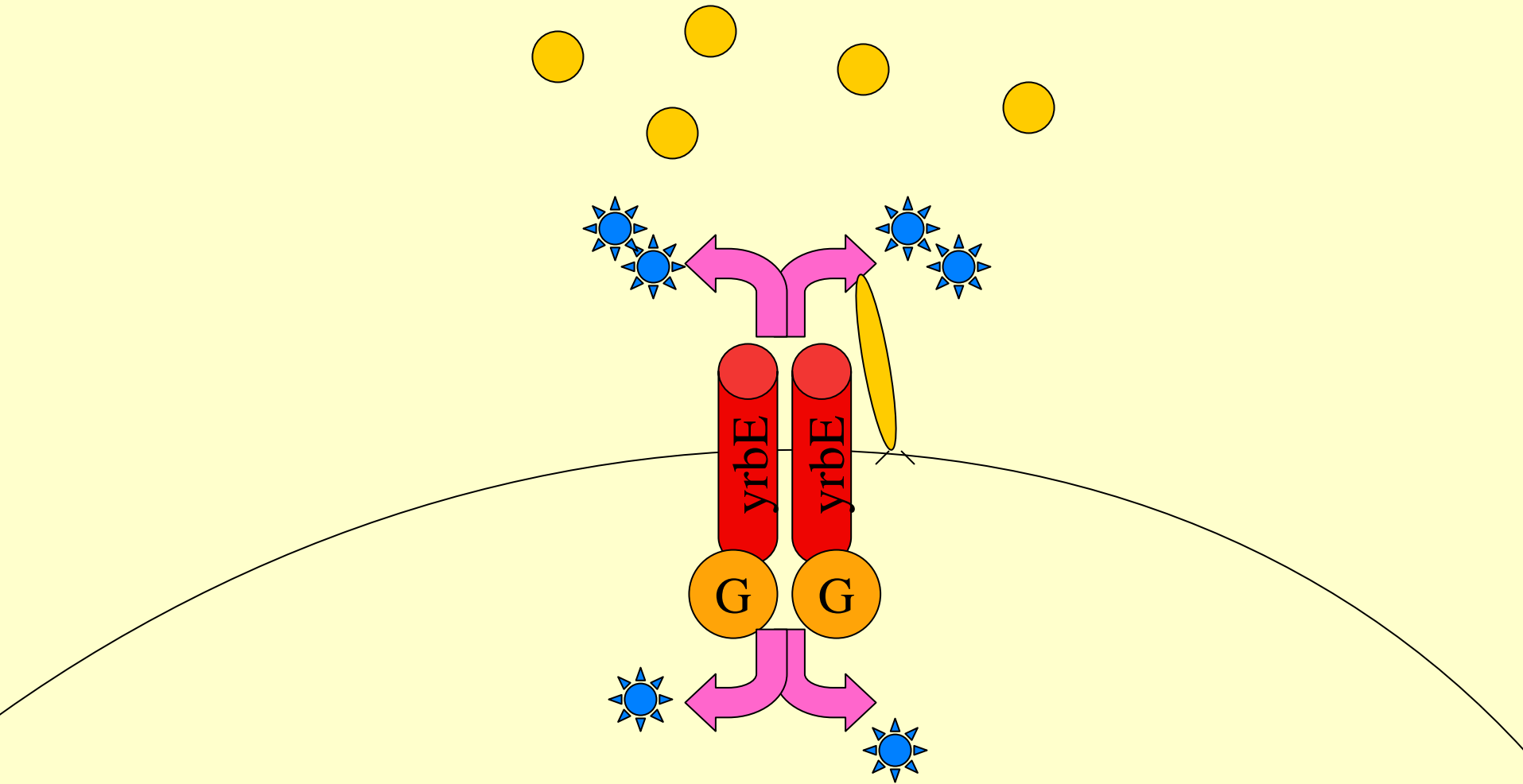
“*mceG*” encodes a NBD subunit that interacts positively with the *mce* loci



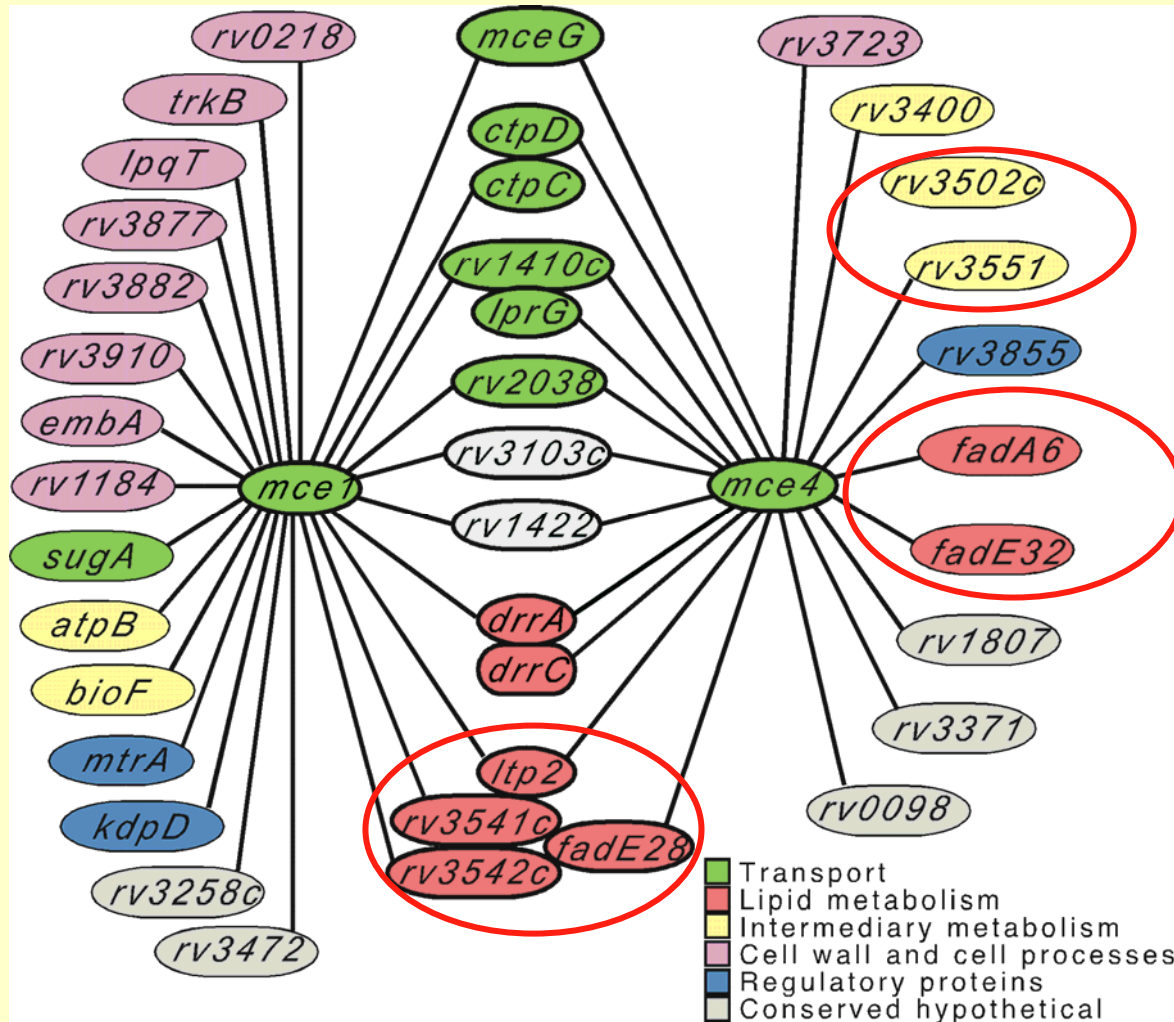
$\Delta mceG$ and $\Delta mce1/4$ mutants have indistinguishable phenotypes



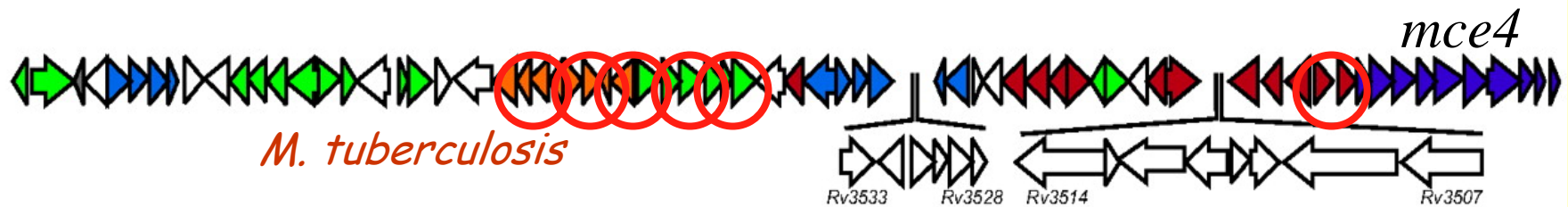
What do they do??



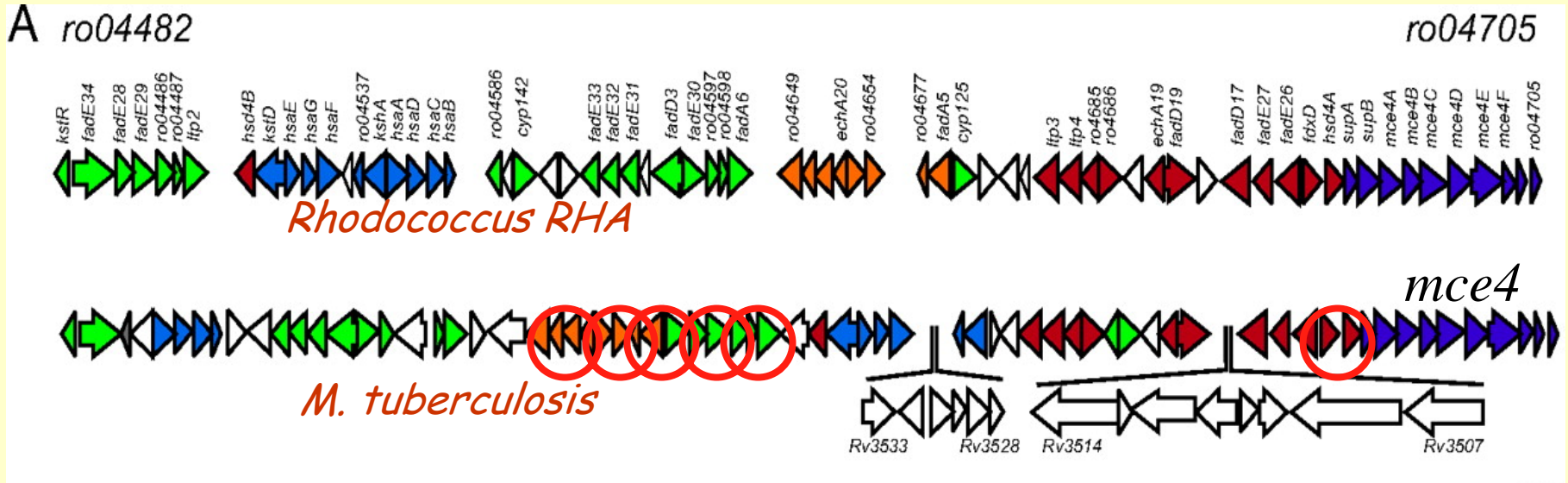
Mce interaction map



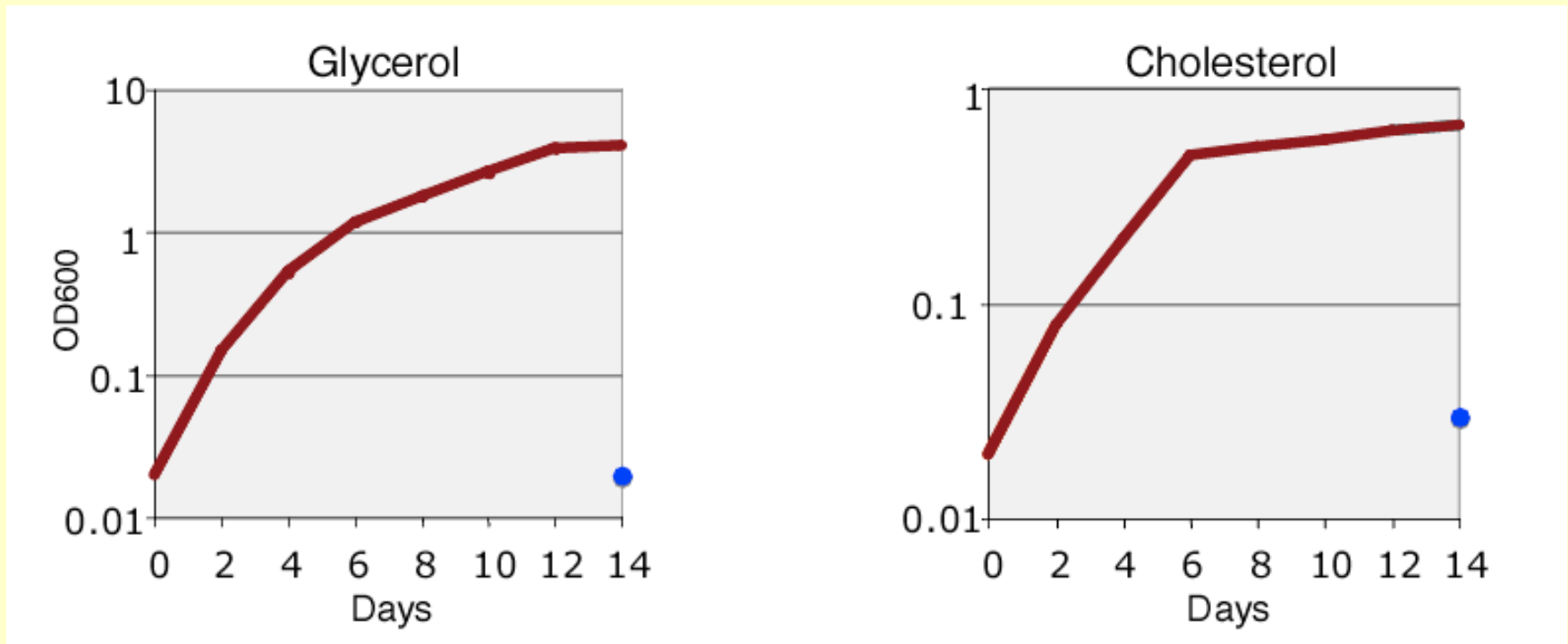
Mce4-interacting genes cluster upstream of the mce4 operon



Mce4-interacting genes cluster upstream of the mce4 operon



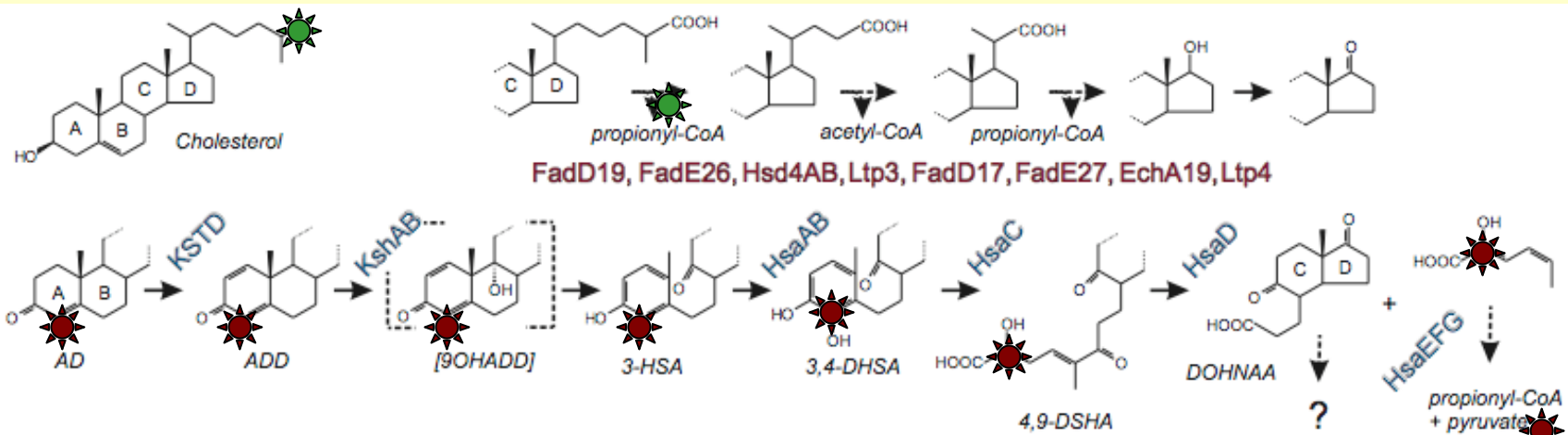
Cholesterol-dependent growth of *M. tuberculosis*



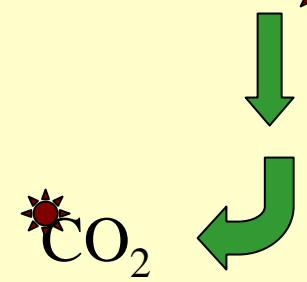
Doubling time = 35.3 hours

38.8 hours

Proposed degradation pathway

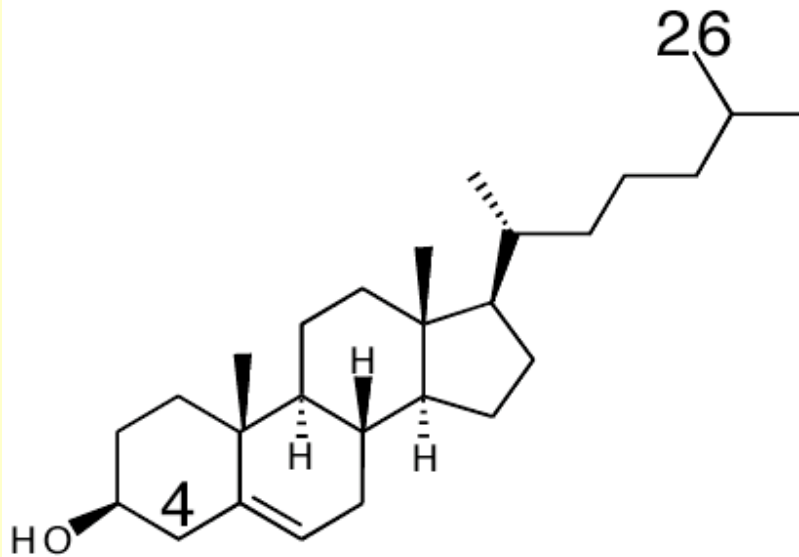


Van der Gieze *et al.* 2007

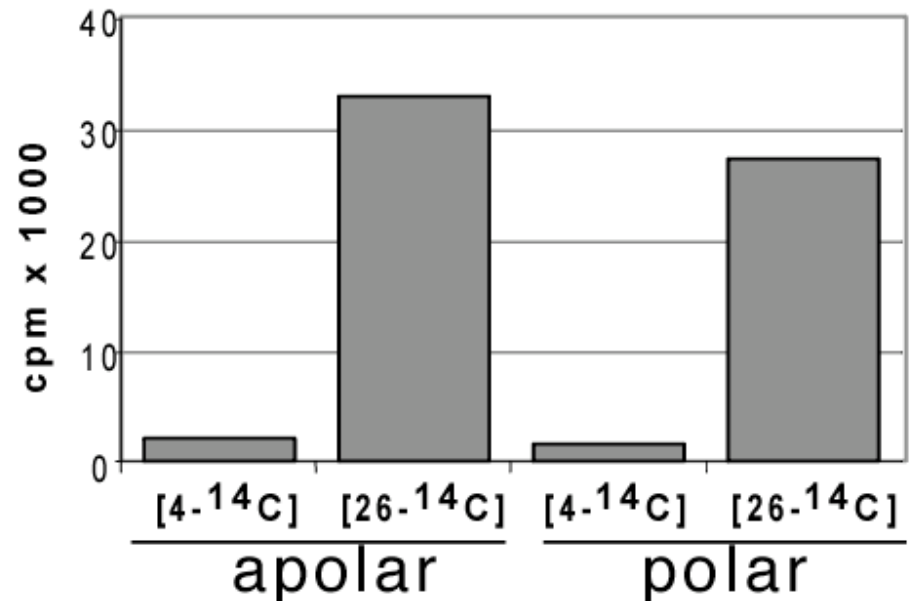


Sidechain carbon is preferentially used for lipid synthesis

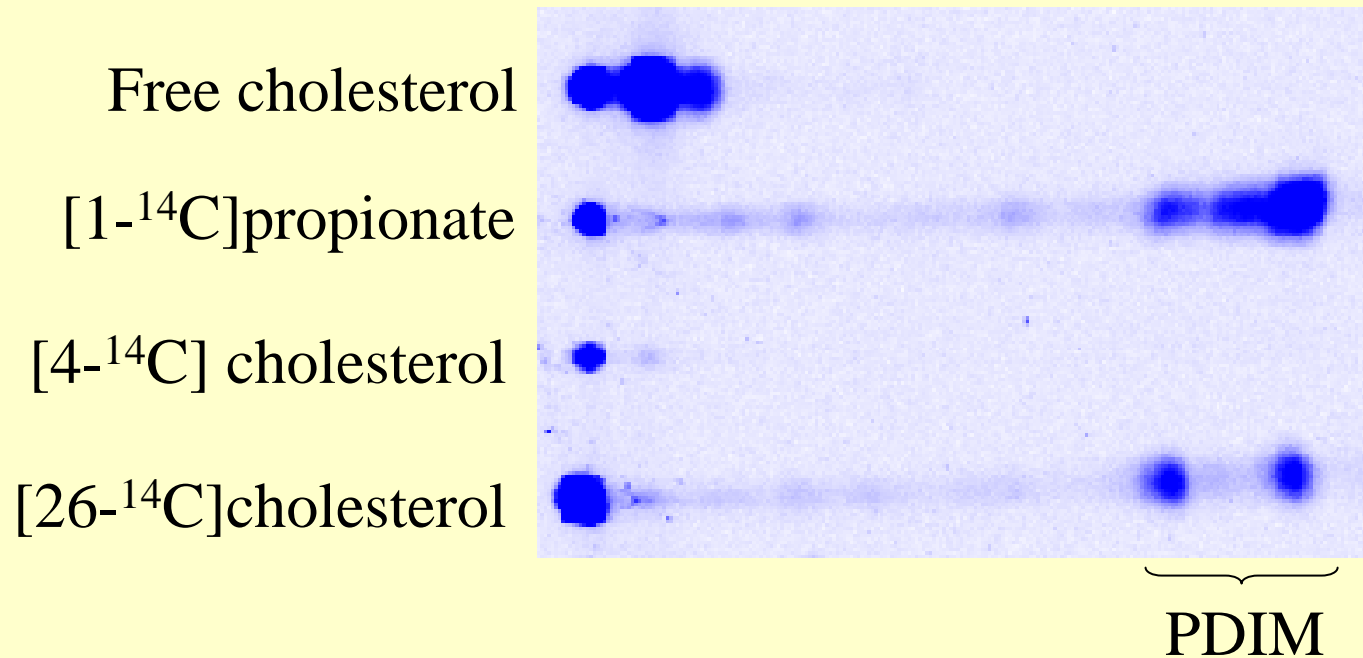
Cholesterol



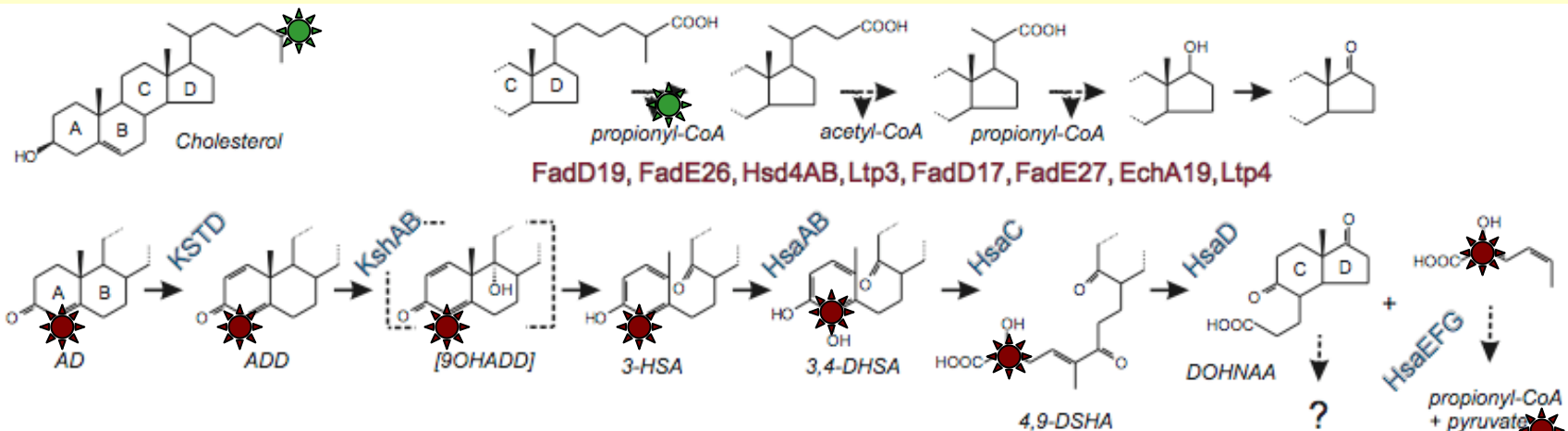
cpm in lipid extracts



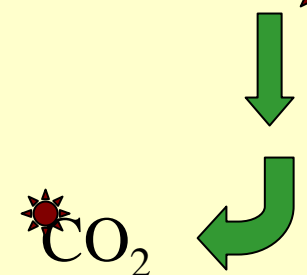
Sidechain carbon becomes incorporated into bacterial lipids



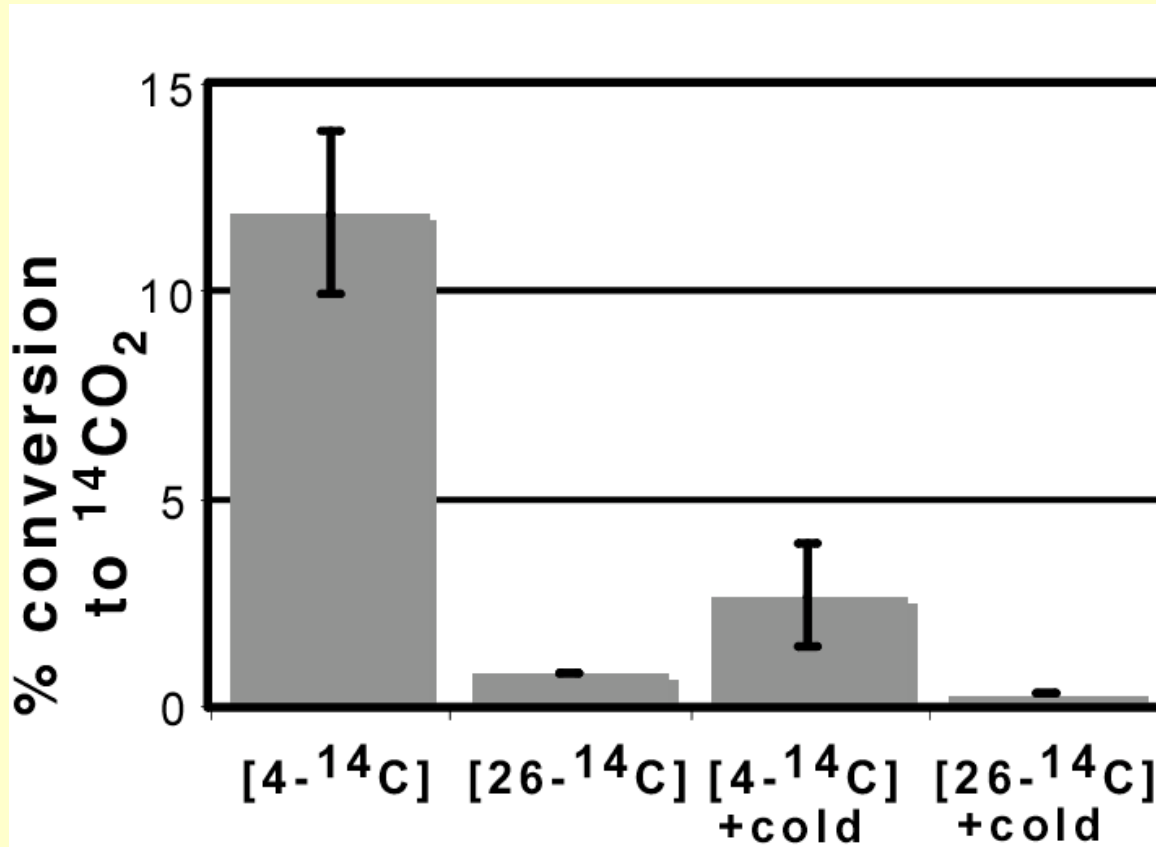
Proposed degradation pathway



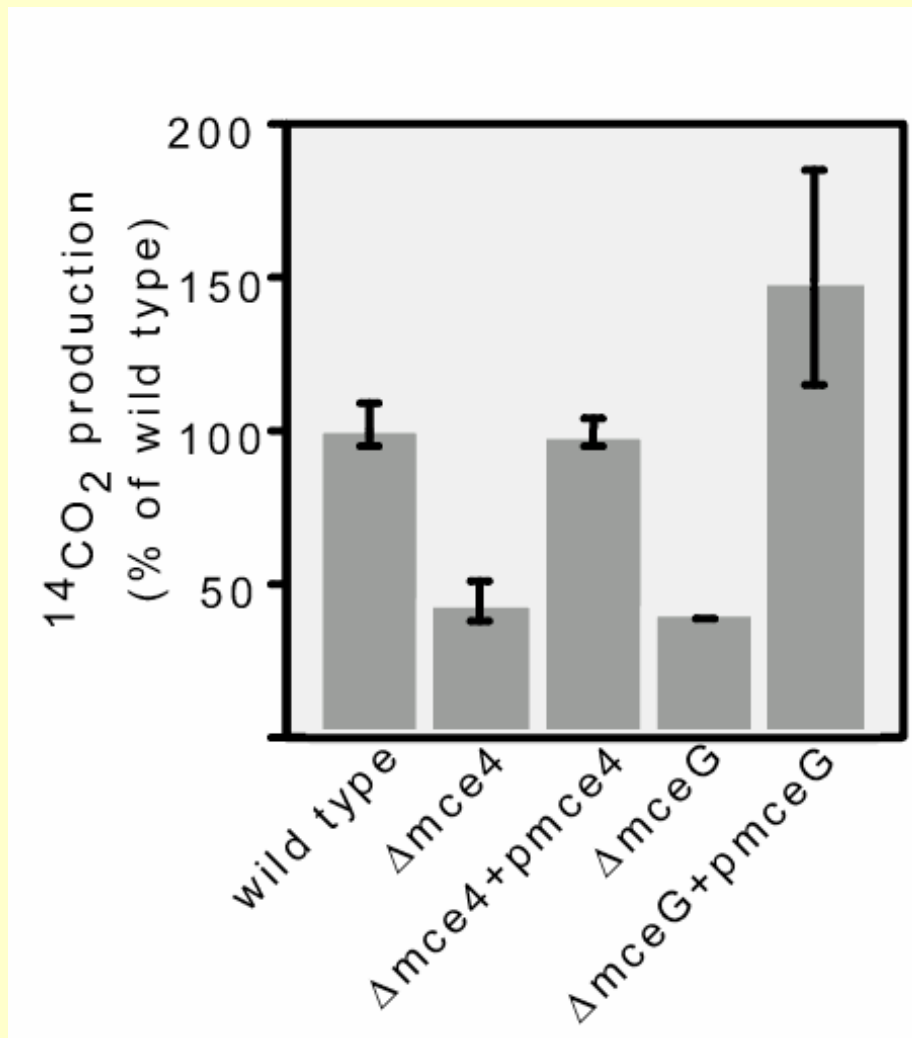
Van der Gieze *et al.* 2007



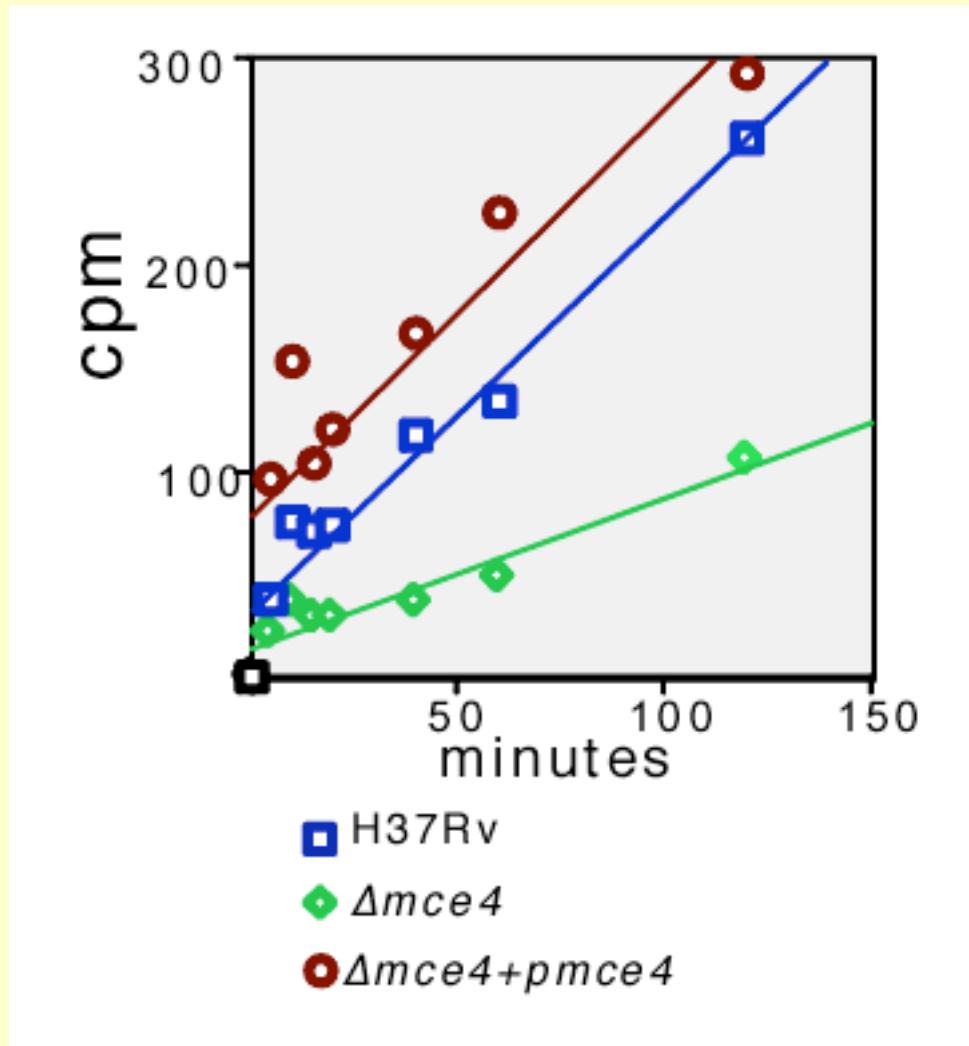
Ring A carbon is preferentially metabolized into CO₂



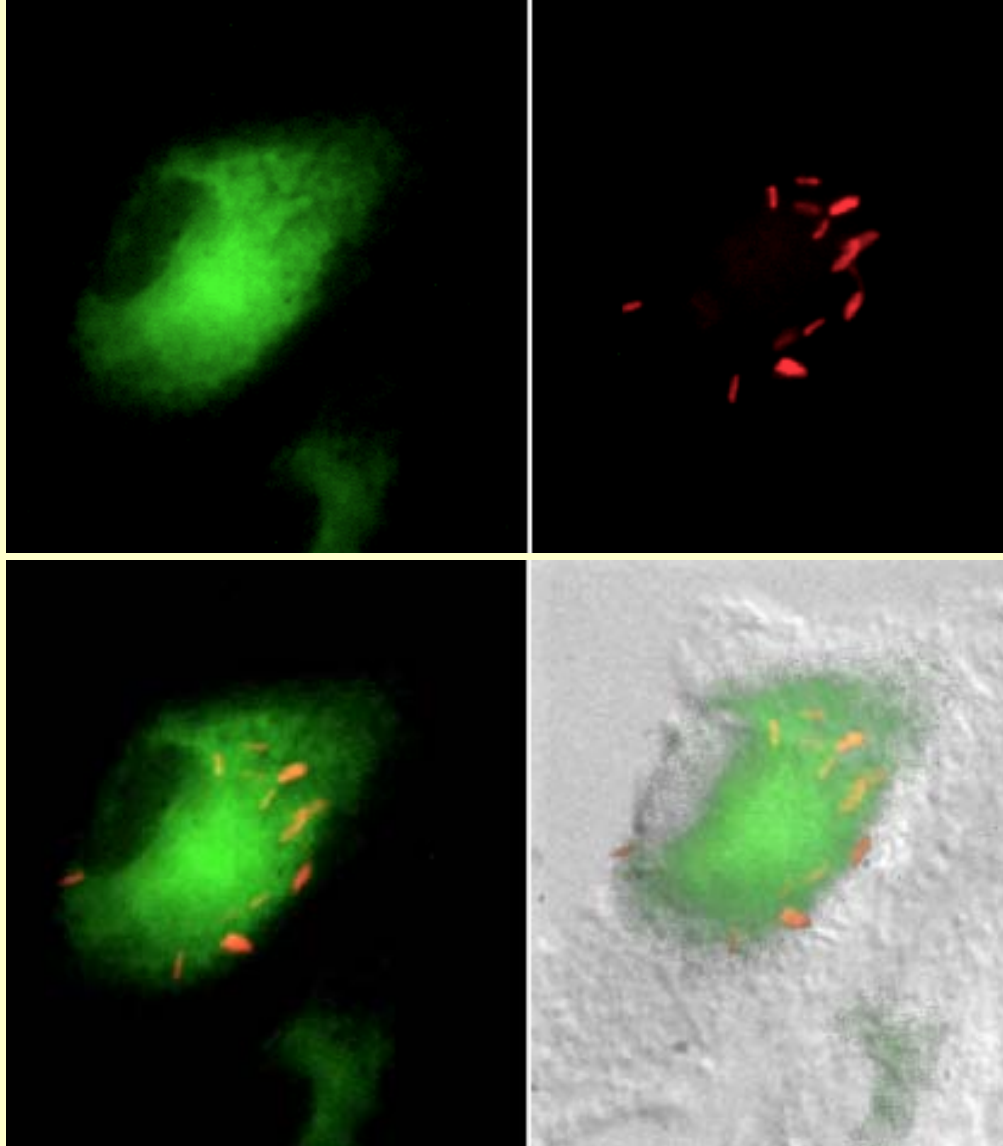
mce4 mutants metabolize cholesterol at a reduced rate



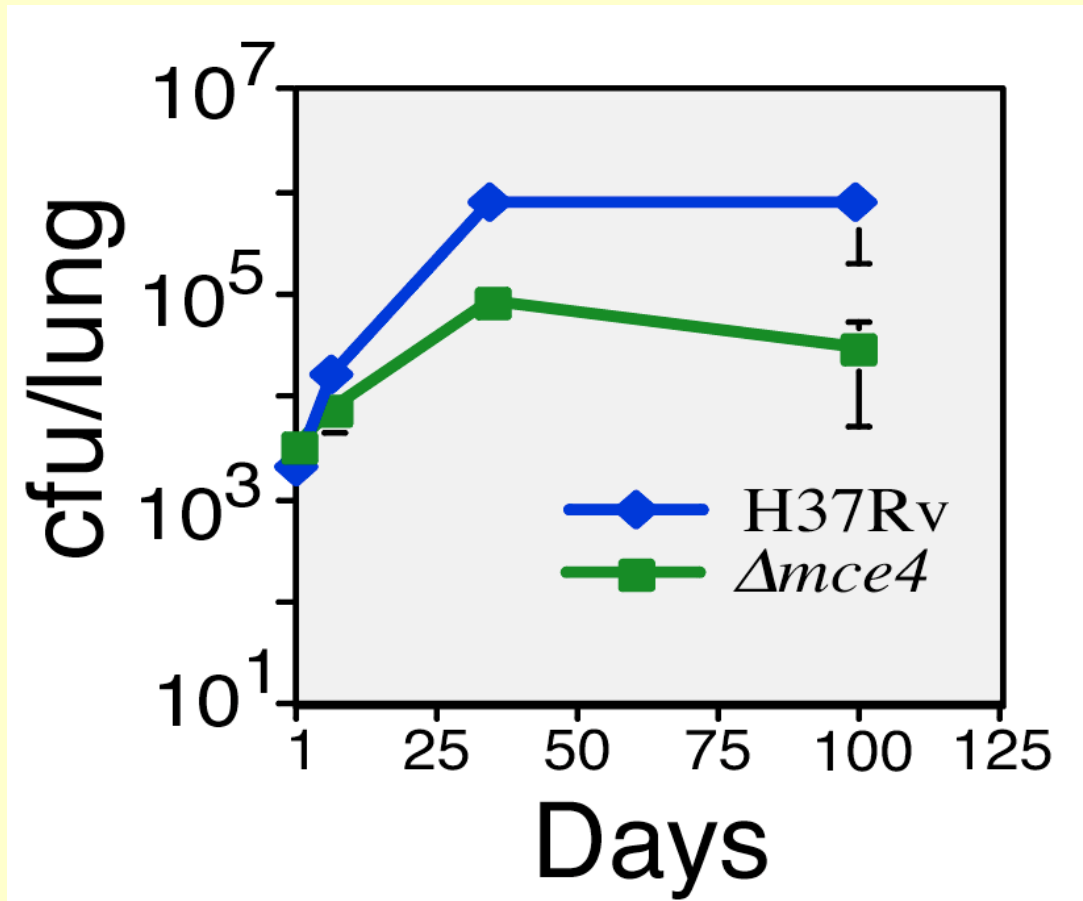
Mce4 is required for cholesterol uptake



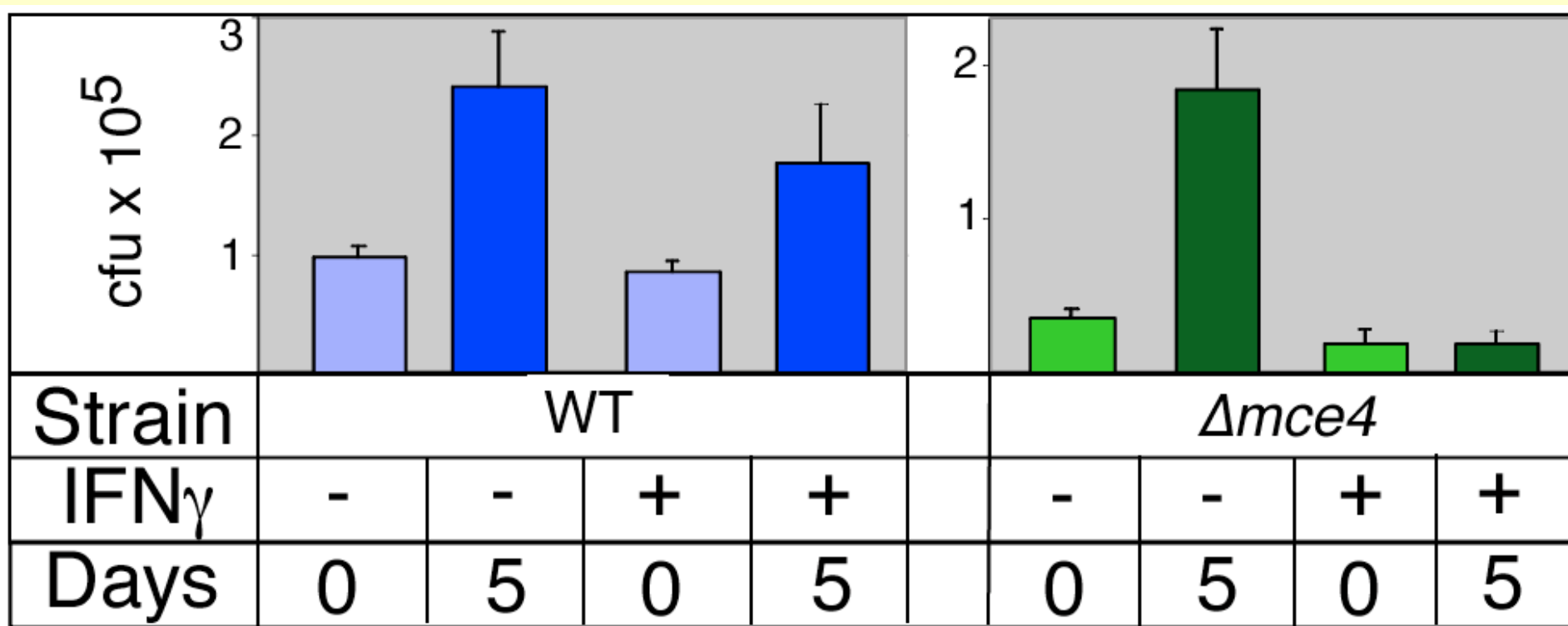
M. tuberculosis grows in
cholesterol-rich regions of the cell



Why is cholesterol catabolism important for chronic persistence?

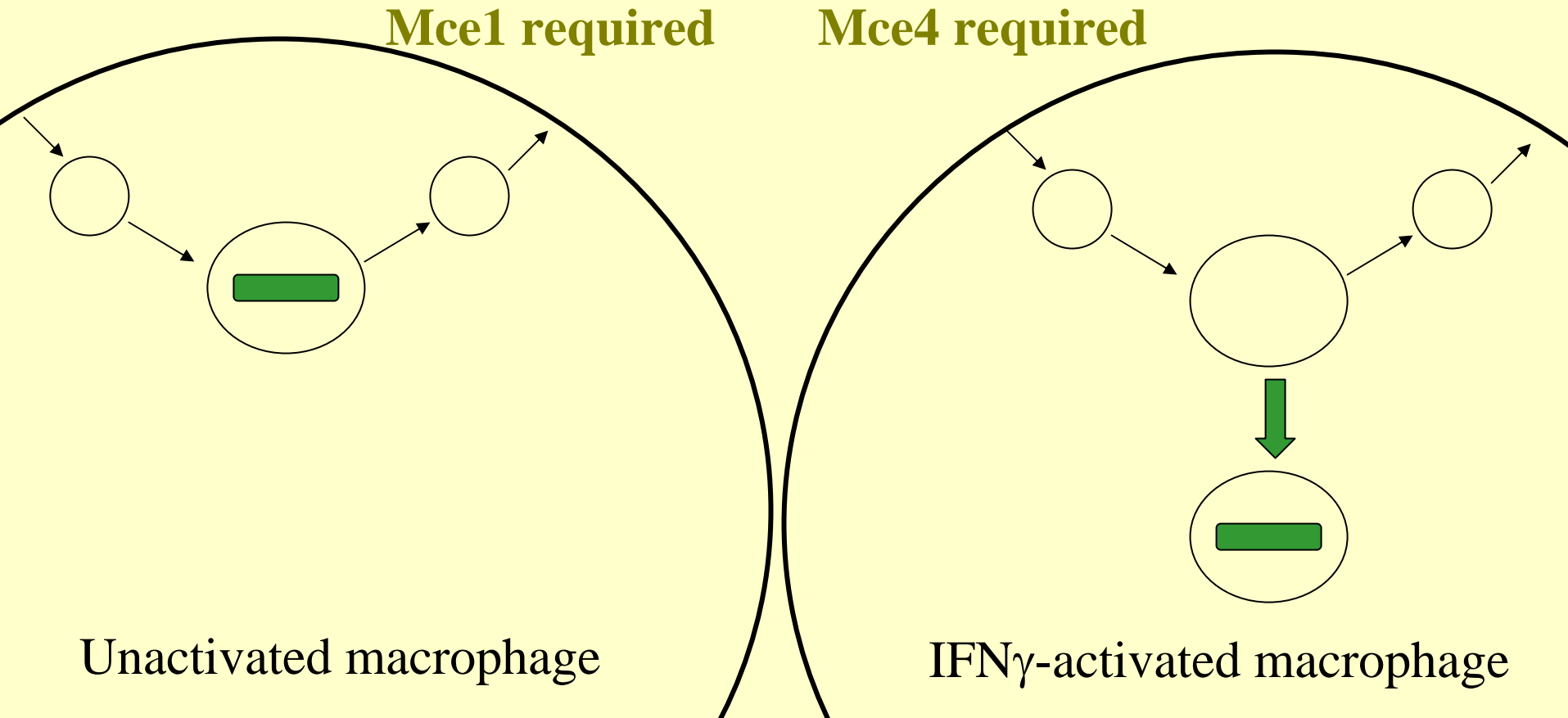


Cholesterol uptake is required for growth in activated macrophages



Model #1:

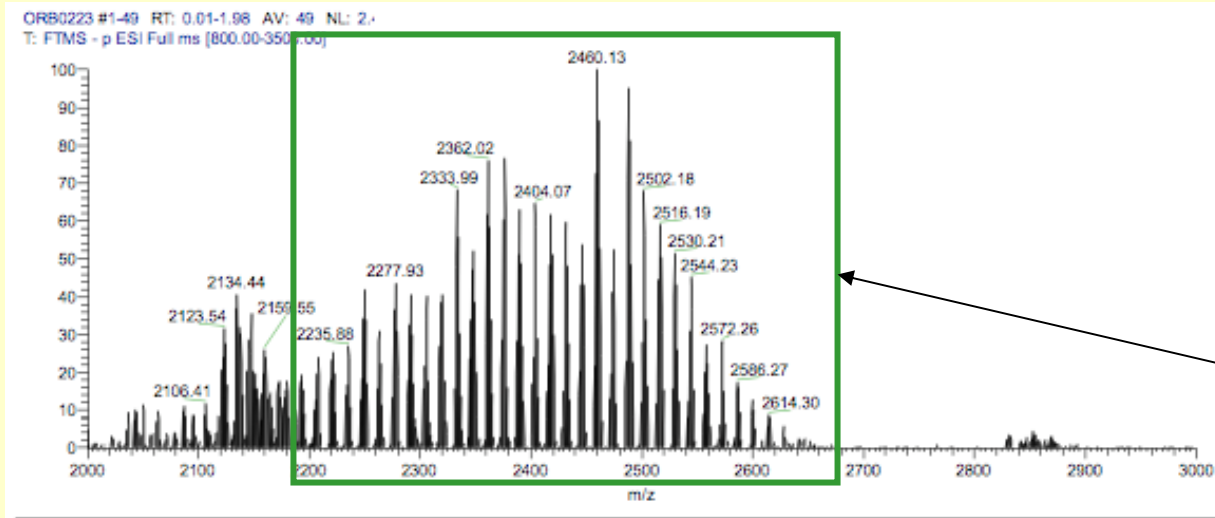
Mce requirements are determined by phagosome maturation



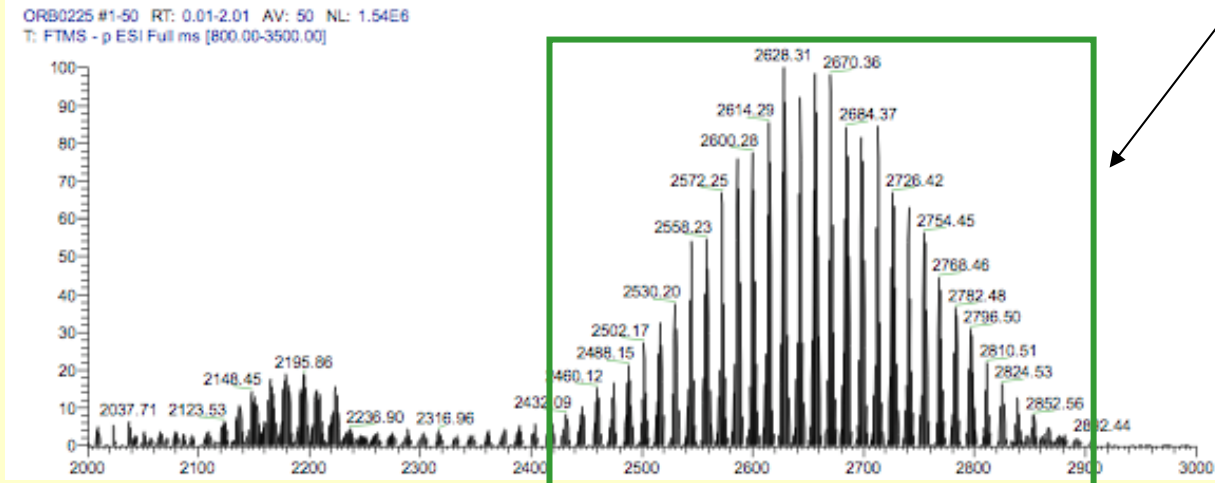
Model #2

Growth on cholesterol alters the cell wall

Glycerol



Cholesterol



SL-1

Other questions

*Why is cholesterol metabolism important for chronic persistence?

Nutrition: Carbon/energy

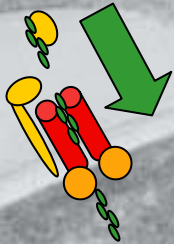
Cell envelope alterations

Miscellany: secondary metabolite synthesis,
manipulation of host sterol metabolism...

*What is the source of cholesterol and how is it extracted from the host?

*Do host cholesterol levels influence TB pathogenesis?

Mce crew



Mce's



Amit Pandey



Jen Griffin



Swati Joshi

Current lab members

- Amit Pandey
- Jennifer Griffin
- Nick Lafave
- Seunghun Baek
- KG Papavinasasundaram
- Chitra Kanchagar

Alums

- Nicole Capite
- Swati Joshi

NIH, Charles H. Hood Foundation,
Pittsfield anti-TB Association

Collaborators

UMass

- Yibin Yang &
Michelle Kelliher
- Greg Martens &
Hardy Kornfeld**
- Kenan Murphy

HSPH

- Eric Rubin

U. of Michigan

- Gabriel Nunez