In Search of Better Cures

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- Undergraduate (Premed) - FSU
- Medical School - University of Alabama at Birmingham
- Residency - Duke University Medical Center
- Service - United States Public Health Service Centers for Disease Control (Atlanta, GA)
- Board Certified - Anatomic and Clinical Pathology

Case 1

“Legionnaires’ Disease”

- Presentation - Malaise, Myalgia, Headache
- 12-24 Hours - Fever, Chill, Prostration
- 24 Hours - Dry cough, Chest pain, X-ray evidence of consolidation

"Legionnaires Disease"
Additional Clinical Features

- Diarrhea
- Encephalopathy
- Relative bradycardia
- Renal failure

(182 LD cases, 28 deaths)

Legionella

- **Common organism in water, especially warm, stagnant water.**
- **May be aerosolized by improperly maintained cooling towers.**
- **Also has been identified in plumbing systems, hot water tanks, and spas.**
- **Source in Philadelphia hotel never identified.**

Treatment

- Legionella is resistant to beta-lactam and aminoglycoside antibiotics.
- Azythromycin, 5 – 10 days is effective and is the treatment of choice in children.
- Other antibiotics require 2 – 3 weeks of treatment. Immunocompromised patients require longer.
- Successful treatment depends on clinical suspicion.

Legionella Pneumonia Outcome

- Resolution
- Superinfection
- Organization with possible permanent impairment
- Death (Approximately 25% overall)

Case 2

Case Presentation: 2011

A 47 year old man at from a homeless shelter goes to a public clinic complaining of chronic cough and not feeling well. He does not remember the last time he held a fulltime job, and has lived on the street in several large cities for at least the last five years. It is difficult to take an accurate history because he seems to be talking to people not in the examination room. The patient remembers being told he had some sort of lung infection about a year ago, but the medicine made him sick and he moved on before taking all of the treatment.

Tuberculosis

- Caused by *Mycobacterium tuberculosis*
- Culture of sputum is the most common method for diagnosis. Several weeks may be required to grow organisms and obtain sensitivity patterns.
- PPD test determines only past exposure to the organism.

Primary Tuberculosis Infection

- Seen as an initial infection, usually in children.
- The initial focus of infection is often just a small subpleural nodule (granuloma), along with hilar lymph node involvement that is more prominent.
- Together, these initial lesions make up the “Ghon complex.”
- In nearly all immunocompetent persons, these granulomas resolve and there is no further spread.

Secondary Tuberculosis Infection

- Seen mostly in adults.
- It can result from a reactivation of a previous infection, particularly when health status declines (as in alcoholics).
- However, most cases probably result from reinfection.
- Typically, the upper lung lobes are most affected, and cavitation can occur.

Miliary Tuberculosis

- When resistance to infection is particularly poor, a “miliary” pattern of spread can occur.
- Grossly, there is a myriad of small millet seed (1-3 mm) sized granulomas, either in lung or in other organs.

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Tuberculosis

- Rarely in immunocompromised patients tuberculosis may cause a severe pneumonia (non-reactive tuberculosis).

Treatment for New Skin Test Conversion

- In general, Isoniazid for nine months.
- Decreases incidence off reactivation and secondary tuberculosis.
- Many factors go into determining whether to recommend treatment.

Secondary Tuberculosis Treatment

Six months total-

- Four drugs for two months
- Two drugs for four months

Long course is required due to slow growth rate of organism.

After treatment there is a 2 – 33% relapse rate.

Incomplete treatment encourages drug resistance.

Case 3

Ebolla

- Abrupt onset
- Severe headache and malaise
- Diarrhea and vomiting
- Maculopapular rash and desquamation
- Gastrointestinal and pulmonary hemorrhage (Hemorrhagic fever)

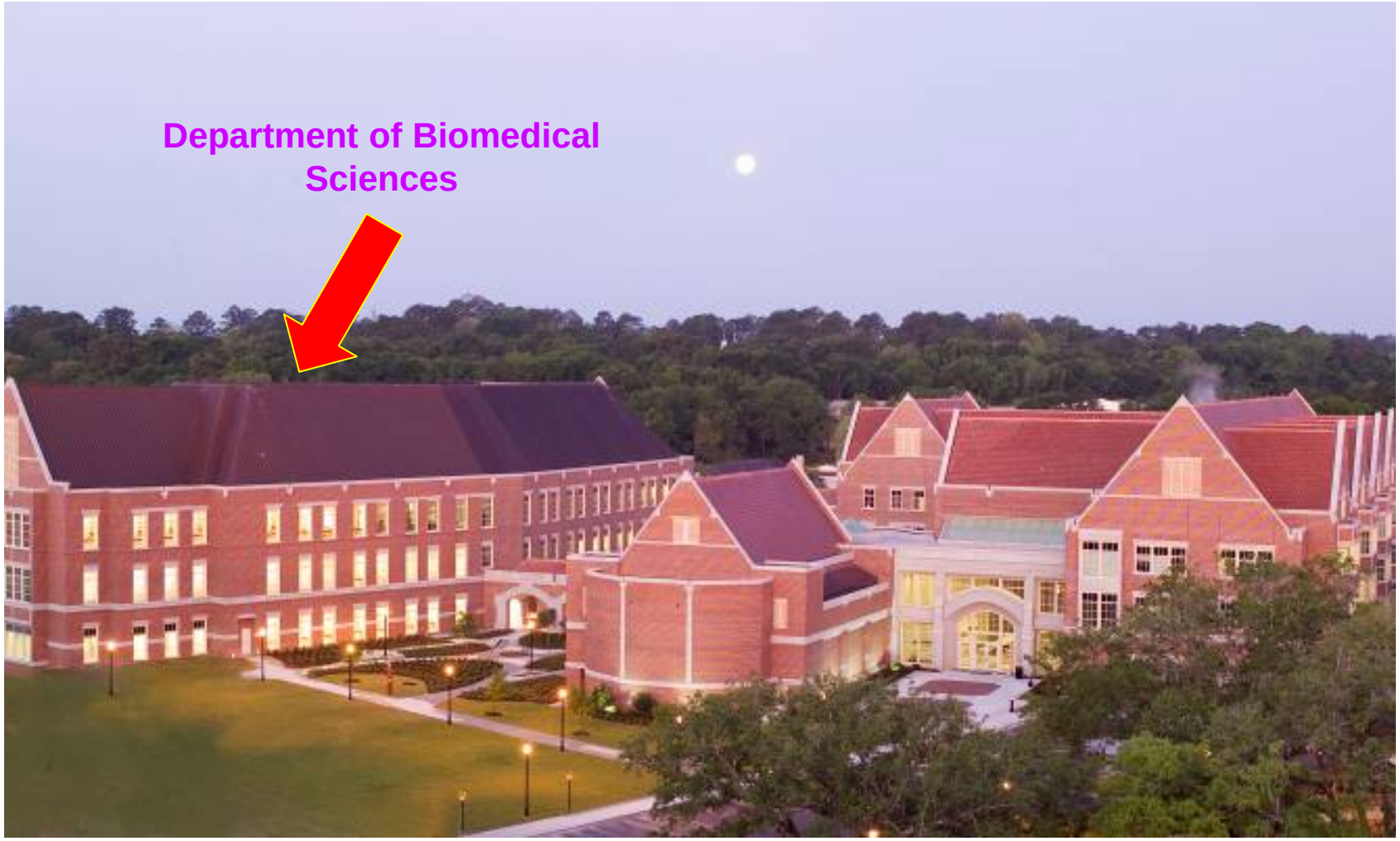
Using molecular approaches to isolate new antibiotics

11/17/2011

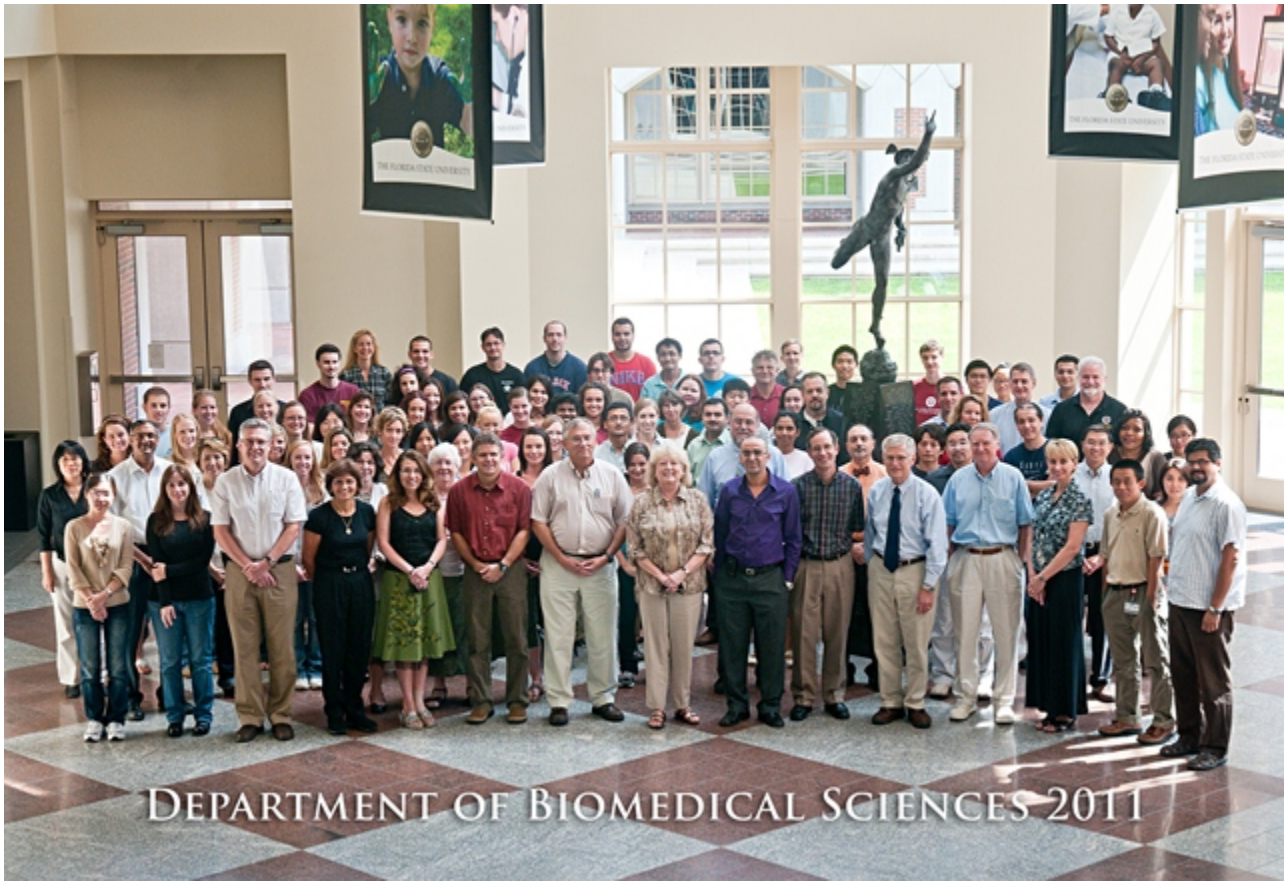
Yanchang Wang

Department of Biomedical Sciences
College of Medicine
Florida State University

College of Medicine
Florida State University



Department of Biomedical Sciences



Medical Student Education

Class of 2012

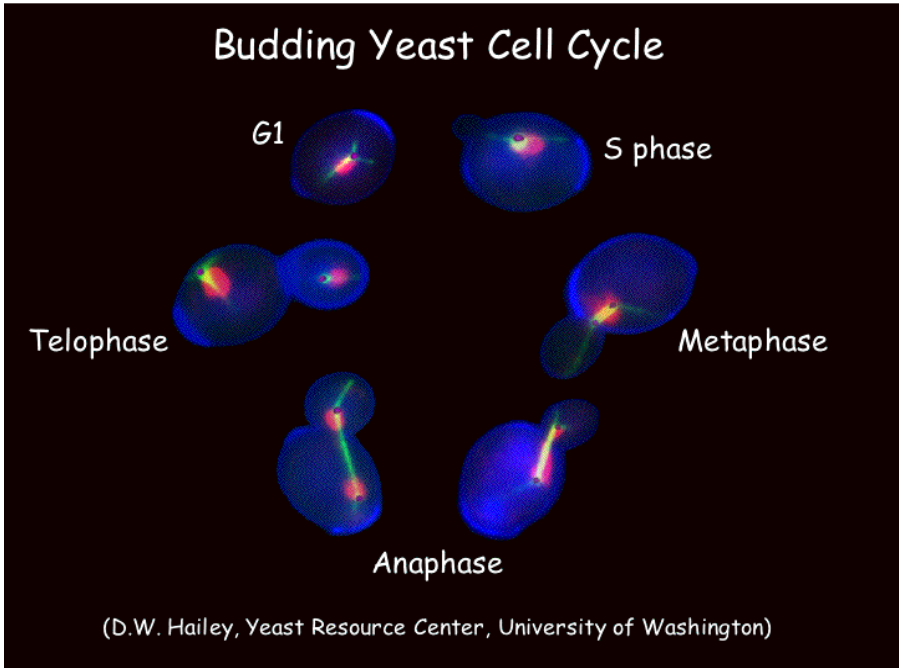
Biomedical Research

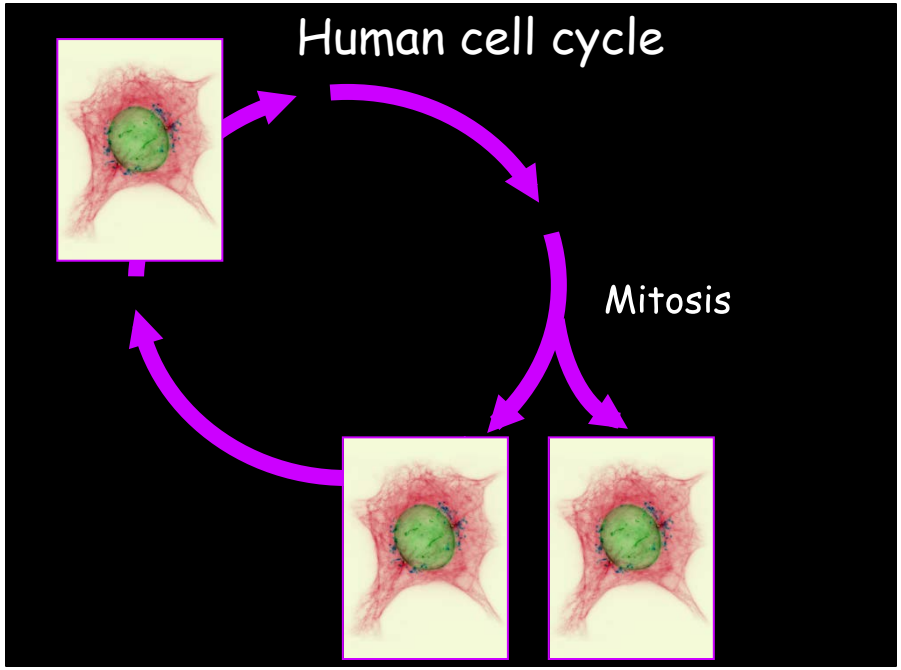
Support:

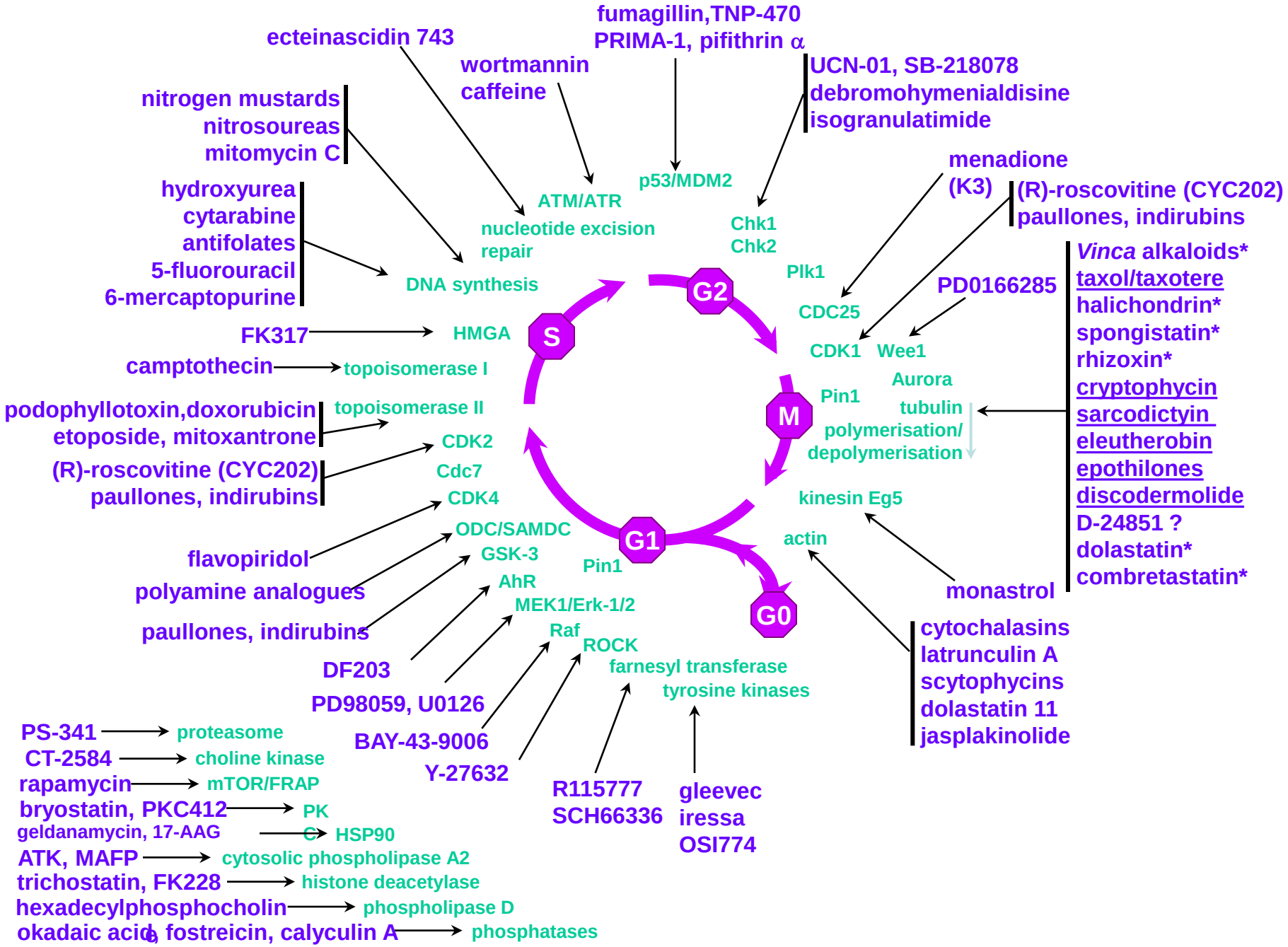
- **Florida State**
- **National Institute of Health**
- **Private foundation (ACS)**

How to justify the support of our research by federal and state money?

Major research interest



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fumagillin,TNP-470
PRIMA-1, pifithrin α
wortmannin
caffeine
UCN-01, SB-218078
debromohymenialdisine
isogranulatimide
nitrogen mustards
nitrosoureas
mitomycin C
menadione
(K3)
(R)-roscovitine (CYC202)
paullones, indirubins
hydroxyurea
cytarabine
antifolates
5-fluorouracil
6-mercaptopurine
ATM/ATR
p53/MDM2
nucleotide excision
repair
Chk1
Chk2
Plk1
DNA synthesis
PD0166285
CDC25
CDK1
Wee1
Vinca alkaloids*
taxol/taxotere
halichondrin*
spongistatin*
rhizoxin*
cryptophycin
sarcodictyin
eleutherobin
epothilones
discodermolide
D-24851 ?
dolastatin*
combretastatin*
FK317
HMGA
camptothecin
topoisomerase I
Aurora
Pin1
tubulin
polymerisation/
depolymerisation
podophyllotoxin,doxorubicin
etoposide, mitoxantrone
topoisomerase II
CDK2
Cdc7
(R)-roscovitine (CYC202)
paullones, indirubins
CDK4
kinesin Eg5
ODC/SAMDC
GSK-3
actin
flavopiridol
polyamine analogues
AhR
Pin1
MEK1/Erk-1/2
monastrol
paullones, indirubins
Raf
ROCK
cytochalasins
latrunculin A
scytophycins
dolastatin 11
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DF203
farnesyl transferase
tyrosine kinases
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PS-341
proteasome
CT-2584
choline kinase
rapamycin
mTOR/FRAP
bryostatin, PKC412
PK
C
geldanamycin, 17-AAG
HSP90
ATK, MAFP
cytosolic phospholipase A2
trichostatin, FK228
histone deacetylase
hexadecylphosphocholin
phospholipase D
okadaic acid, fostreicin, calyculin A
phosphatases
G2
S
M
G1
G0

Modern biotechnology and drug screen

From an idea to new anti-TB antibiotics

Yanchang Wang (Florida State University)

Shuyi Si (Chinese Academy of Medical Sciences)

Idea→ Screen strategy → Results

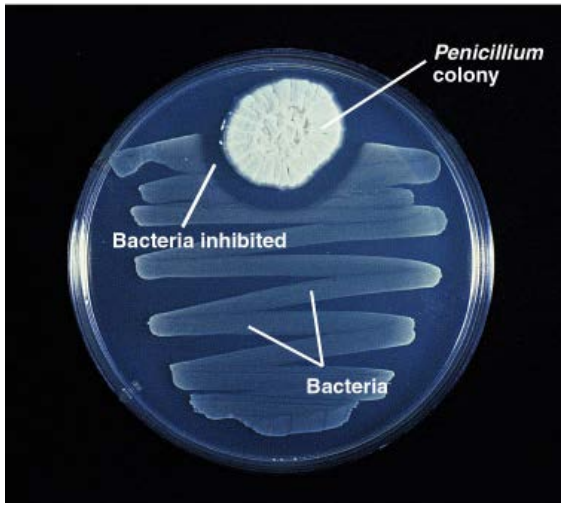
Antibiotics

on the morning of Friday, Sept. 28 1928
• Alexander Fleming observed the killing of staphylococci by a fungus (Penicillium notatum)

• Florey & Chain purified Penicillin (1940)
- Three shared Nobel prize in1945

• First use in a patient: 1942

• World War II: saved 12-15% of lives



In 1999, *Time* magazine named Fleming one of the 100 most important people of the 20th century for his discovery of penicillin.

Antibiotics

- Selman Waksman - Streptomycin (1943)

– active against all Gram-negatives

– first antibiotic against *M. tuberculosis* (TB)

– extracted from Streptomyces

– 20 other antibiotics, neomycin, actinomycin

Sleman Waksman
Nobel prize 1952

Waksman institute of Microbiology

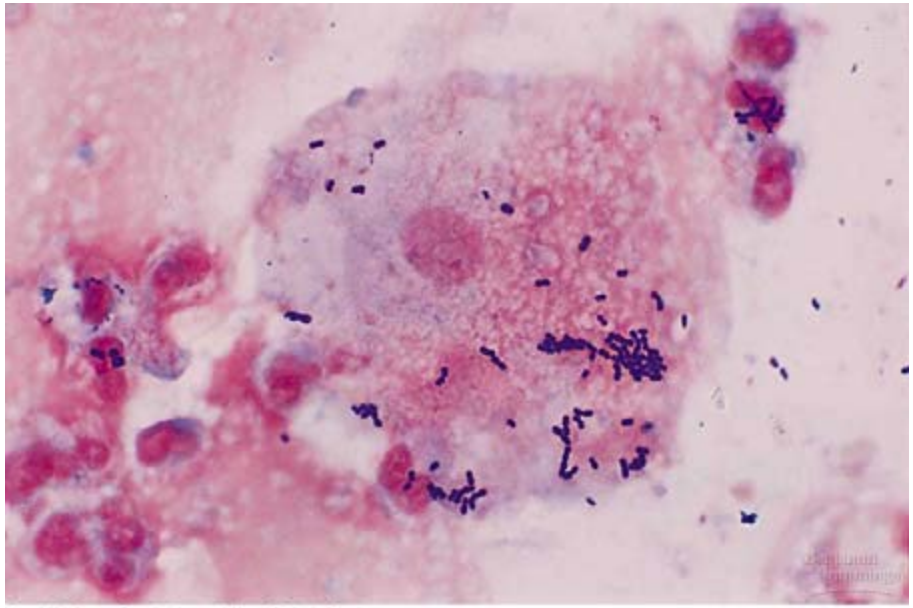
Antibiotic

- **Antibiotics are drugs used to treat infections caused by bacteria, fungi, and virus.**

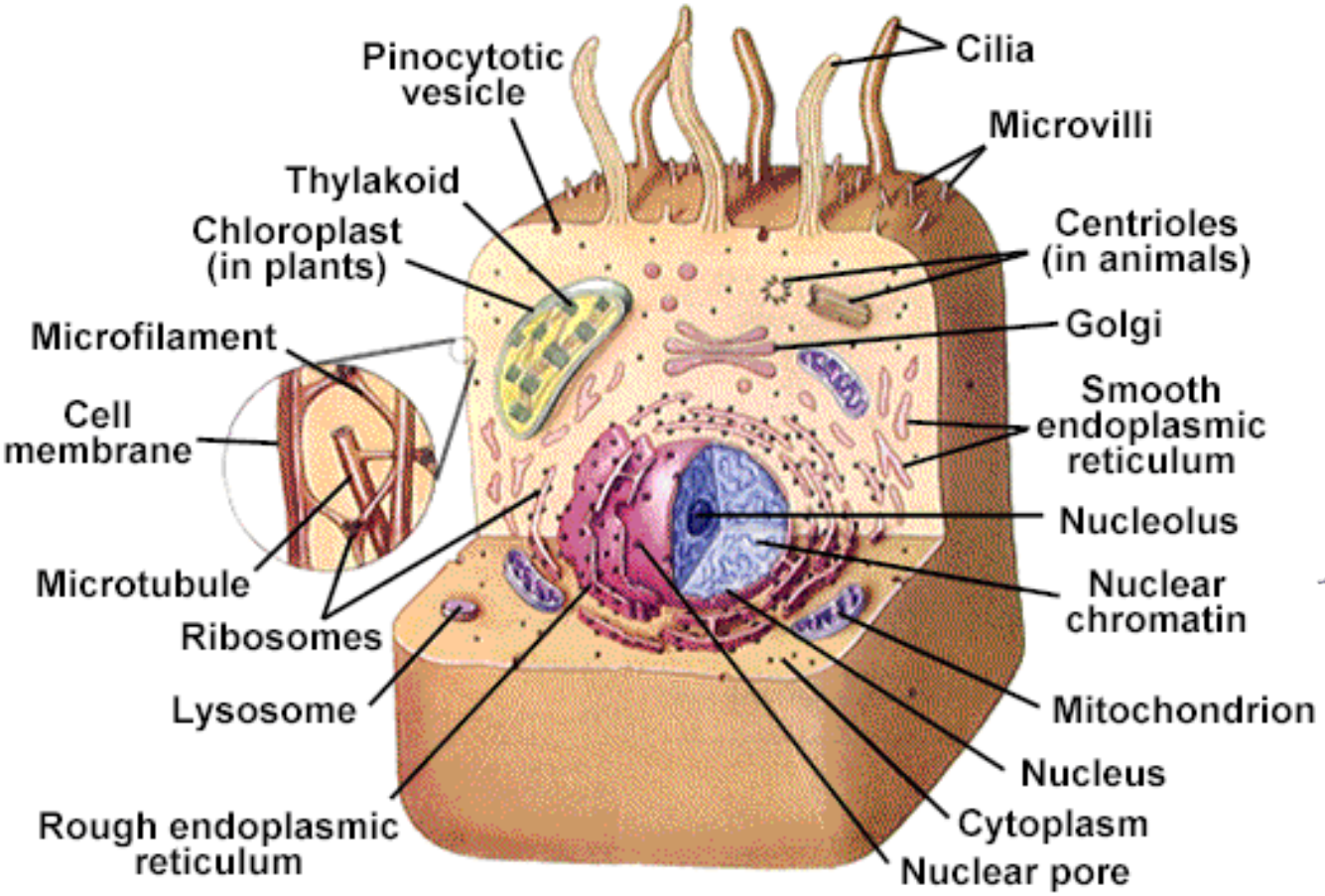
- **Antibiotics could be products of microorganisms and synthetic or semi-synthetic compounds**

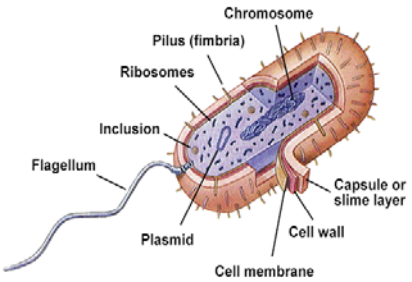
- **Key: it needs to kill the microbial cell and not be toxic to normal healthy human cells.**

Bacteria and human cells



Principle of the isolation of antibiotics

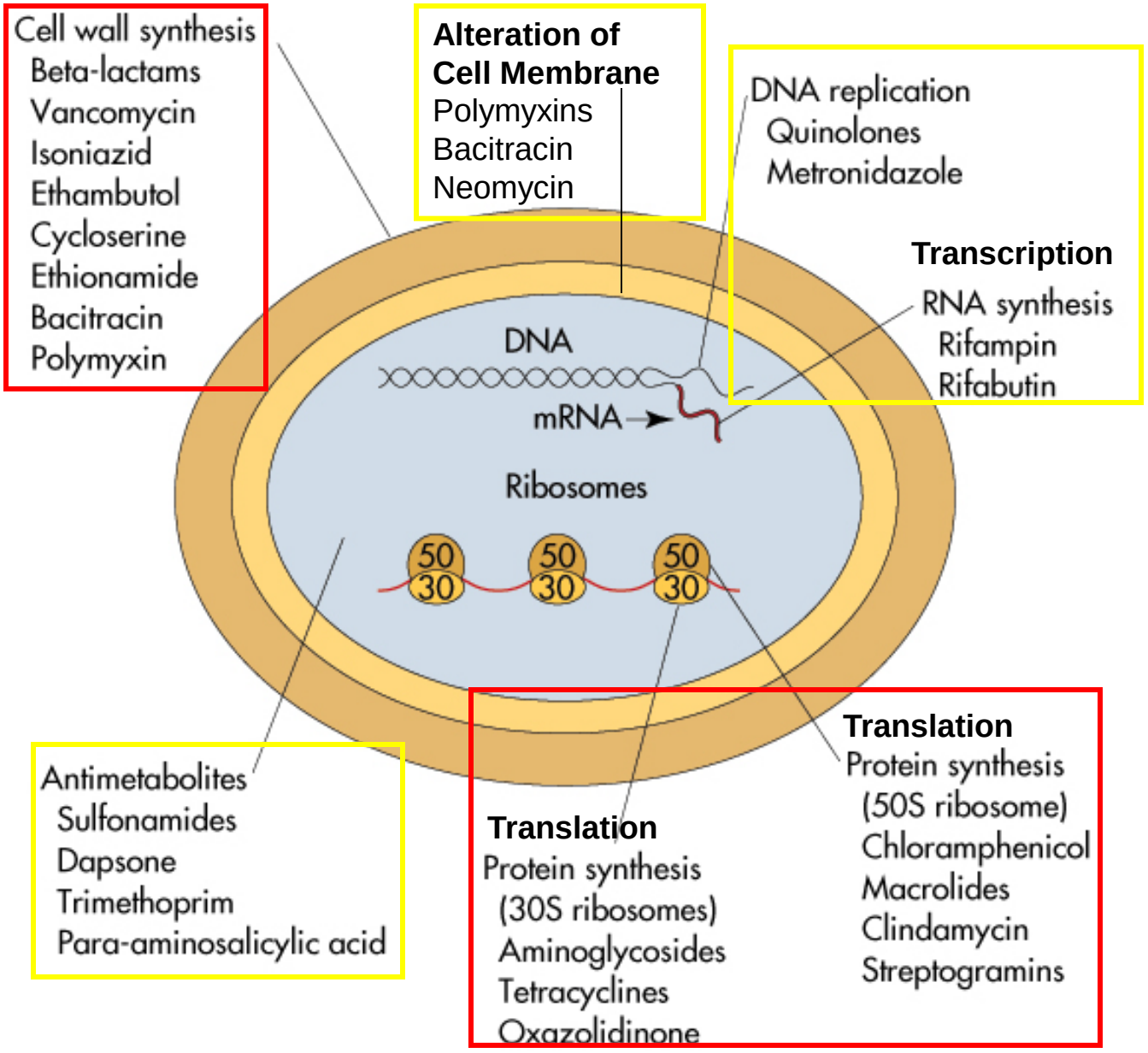




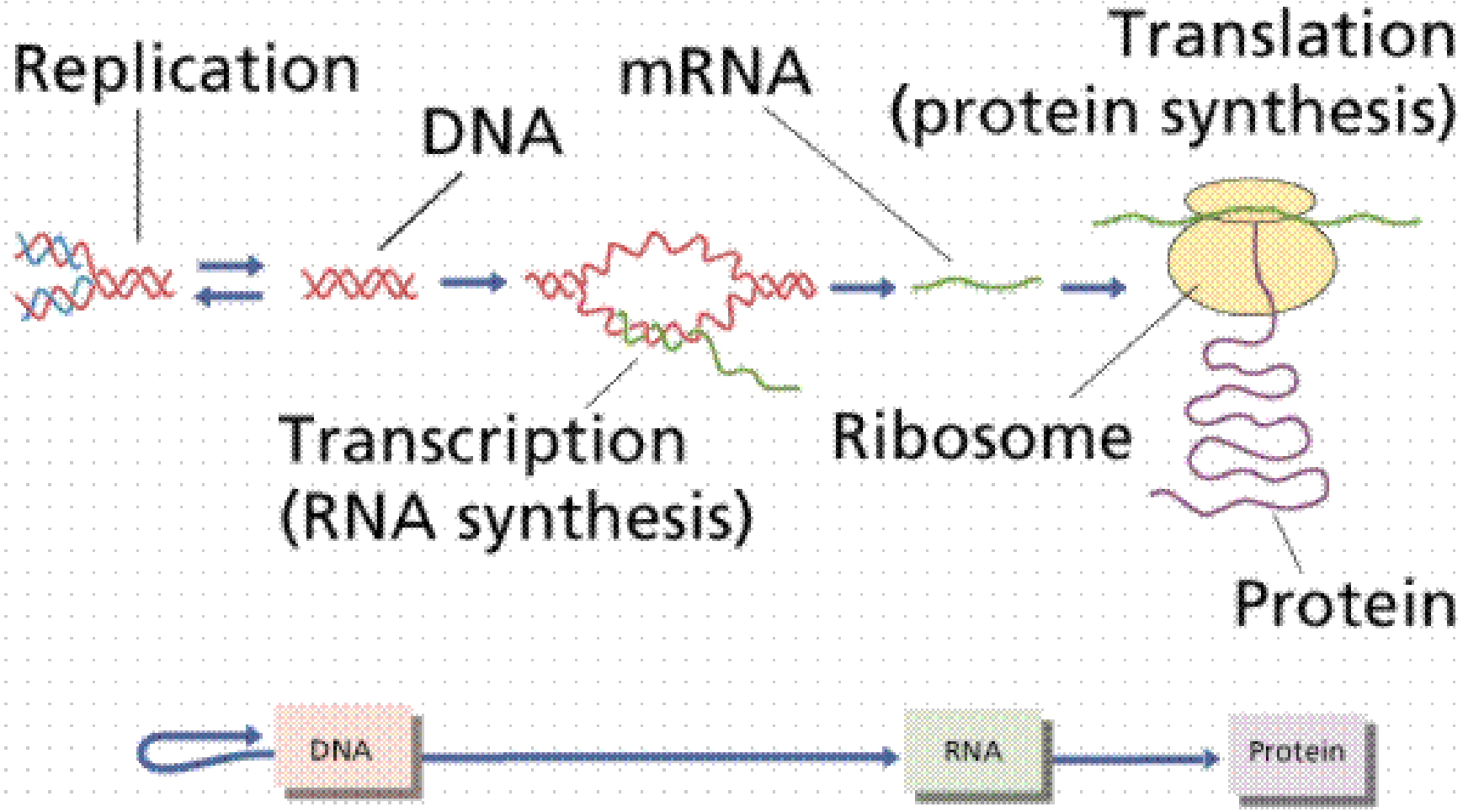
The key difference between human cells and bacteria

Characteristic	bacterial Cell	human Cell
size	averge size 1-10 µm	averge size 10-100 µm
nucleus	nucleoid (no membrane)	membrane bound
chromosomes	single circular loop of naked DNA	linear, arranged with histones
organelles	absent	present, vary with cell function
ribosomes	present as smaller 70S	present as larger 80S
cell wall	present	absent

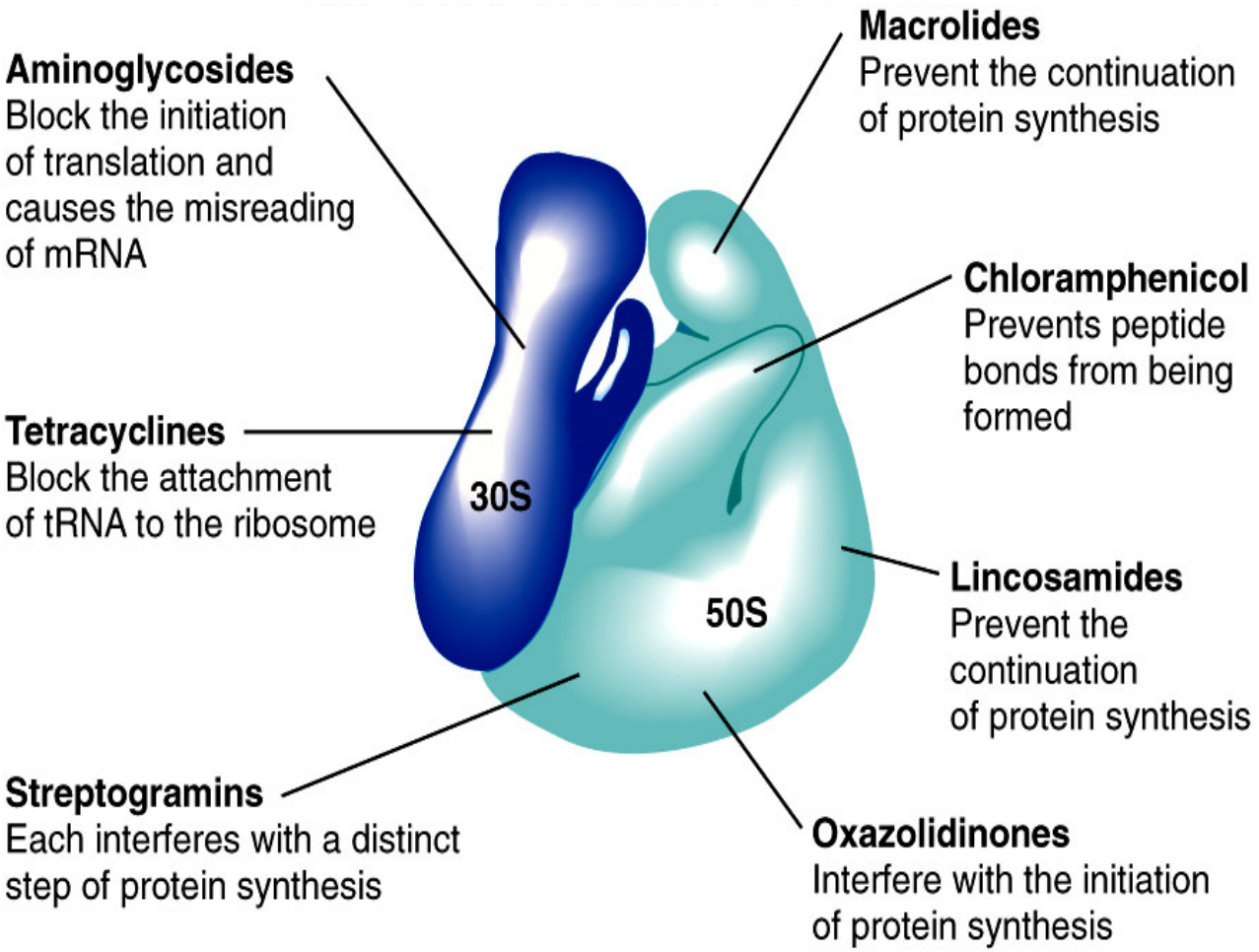
Antibiotic Mechanisms of Action



Ribosome and protein synthesis



Ribosome as a target for antibiotics

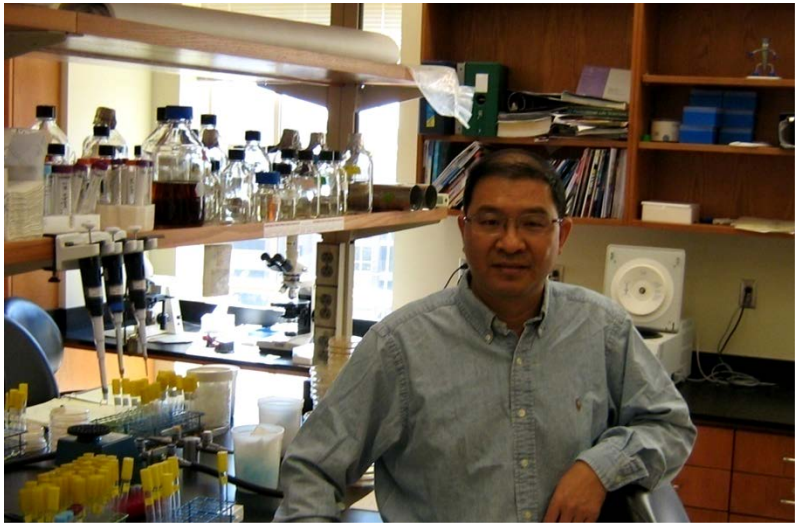


Streptomyces and antibiotics

Cefoxitin
Chloramphenicol
Daptomycin
Lincomycin
Neomycin
Puromycin
Rifamycin
Streptomycin
Tetracycline
Vancomycin

Old and modern days for biomedical research

1989



2011

- New technology in molecular biology
- Genome database
- Compound library

New anti-tuberculosis (anti-TB) antibiotics are needed

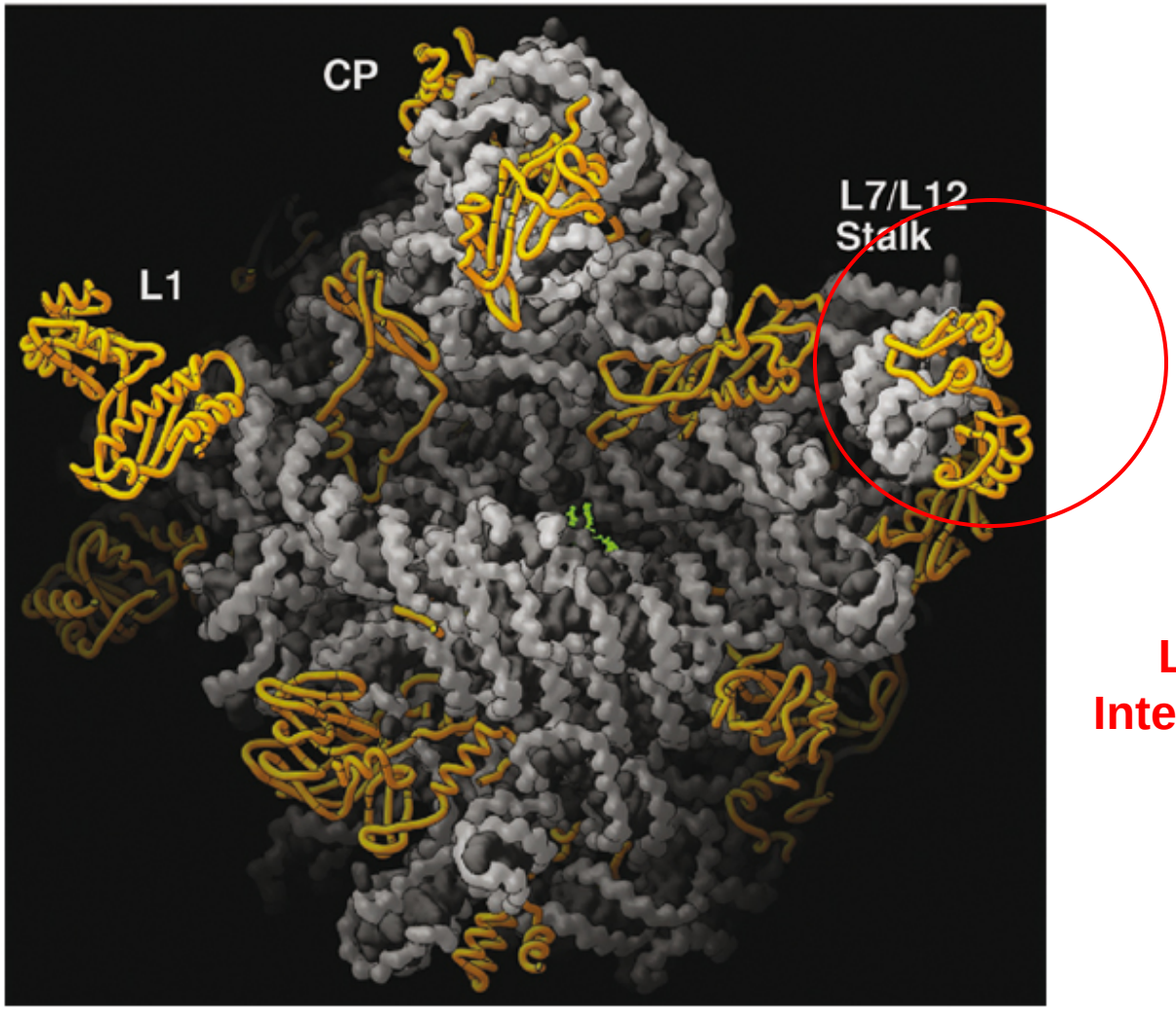
- **2 million people die of TB**
- **Available antibiotics:**

  Rifampin: RNA synthesis, 1967
  Isoniazid: Cell wall, 1952

- **Problems:**

  More people are infected (AIDS)
  The appearance of drug resistant strains

50S Structure of Ribosome



L12 protein
Interacts with L10

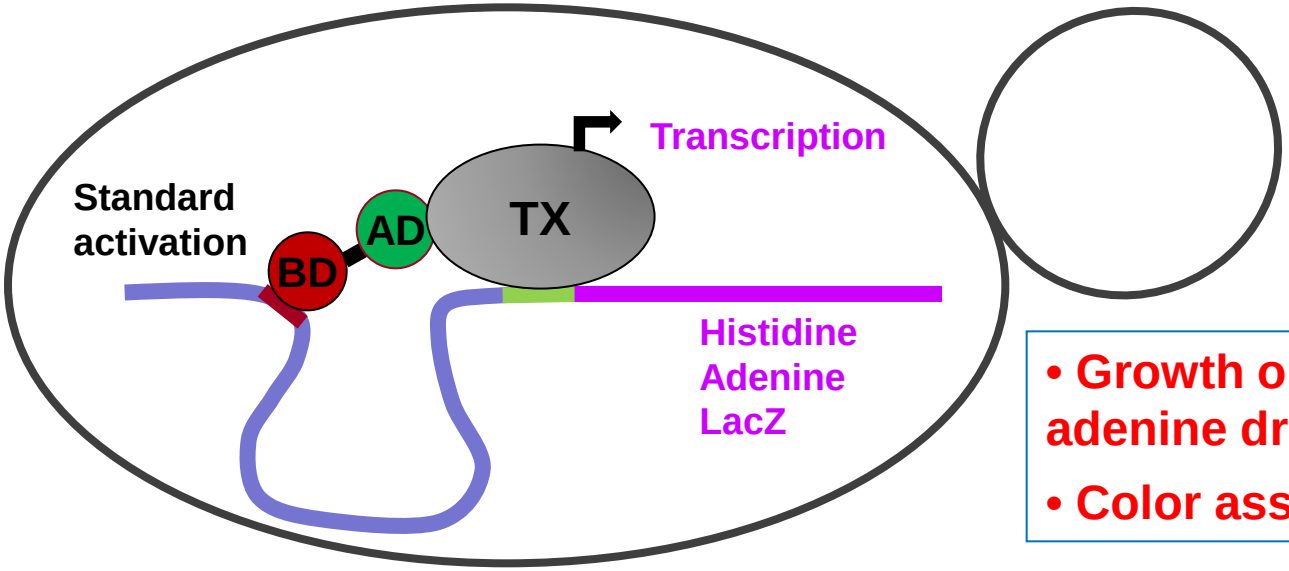
Bacteria and human cells show similar but different L12 protein sequence

L12 protein sequence alignment

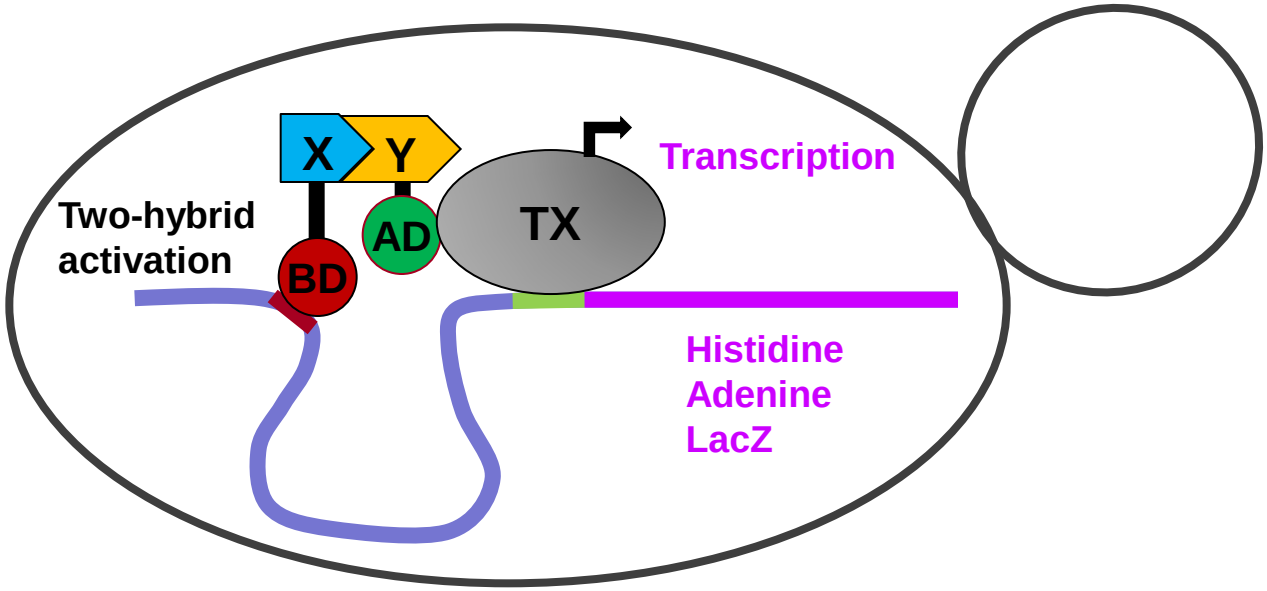
```
Human    MPPKFDPNEIKVVYLRCTGGEVG---ATSALAPKIGPLGLIEVVPSASALIIKALKEPPR
TB       -MAKLSTDELLDAFKEMTLLELSDFVKKFEETFEVTAAAPVAVAAAGAAPAGAAVEAAEE
           .*:..:*:  .: . *  *:.    .   : :: . . : *..:.:*    *:: . .

Human    DRKKQKNIKHSGNITFDEIVNIARQMRHRSLARELSGTIKEILGTAQSVGCNVDGRHPHD
TB       QSEFDVILEAAG---DKKIGVIKVVREIVSGLGLKEAKDLVDGAPKPLLEKVAKEAADE
         : : :  :: :*    *: :.: : :*.   .  *. : . : *:.:.:  :*  . ..:

Human    IIDDINSGAVECPAS
TB       AKAKLEAAGATVTVK
           .::*:...  ...
```

We can see protein-protein interaction

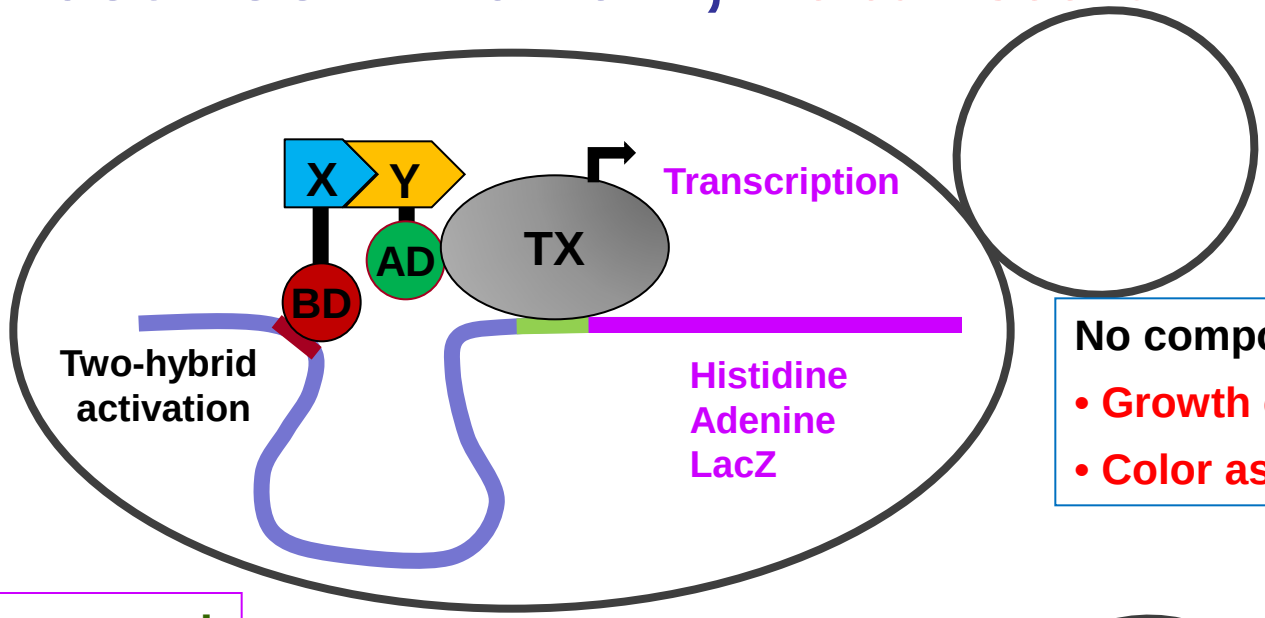
-Yeast Two-hybrid Assay



- Growth on Histidine and adenine dropout plates
- Color assay for LacZ



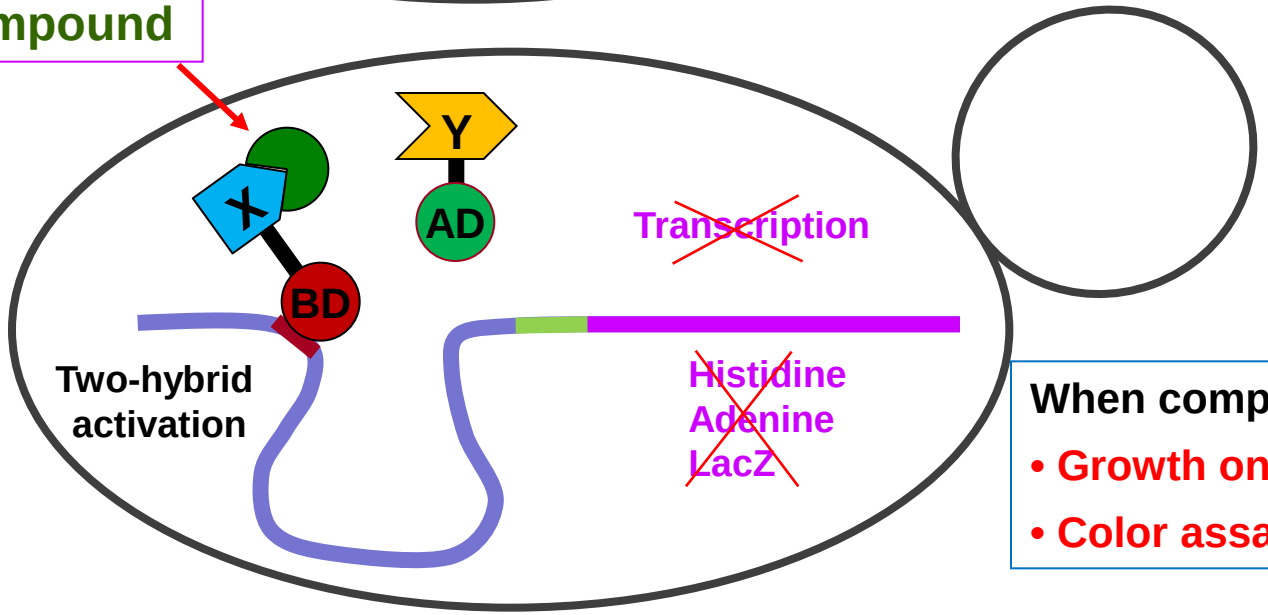
If a compound disrupts the interaction between X and Y, we can see it!



No compounds:

- **Growth on His$^-$ Ade$^-$: Yes**
- **Color assay LacZ: Yes**

A compound



When compounds present:

- **Growth on His$^-$ Ade$^-$: No**
- **Color assay LacZ: No**

Isolation of new anti-TB antibiotics targeting ribosomal L12-L10 interaction

Wang: Tallahassee, US
Knows yeast two-hybrid

+

Si: Beijing China
Knows TB ribosome

Screen procedure

1. To see L12-L10 interaction.

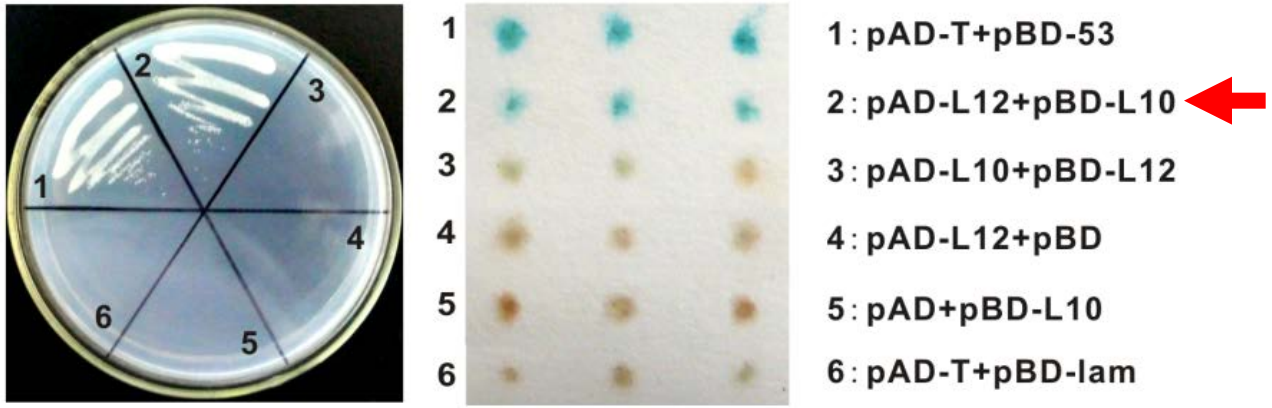
2. To isolate compounds that disrupt L12-L10 interaction.

3. These compounds will kill TB.

4. These compounds should be less toxic to human cells.

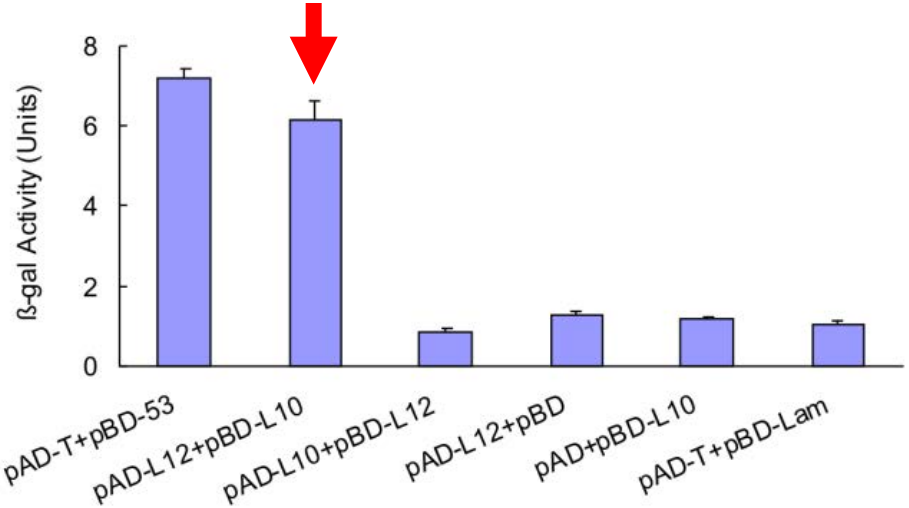
To see L12-L10 interaction

B

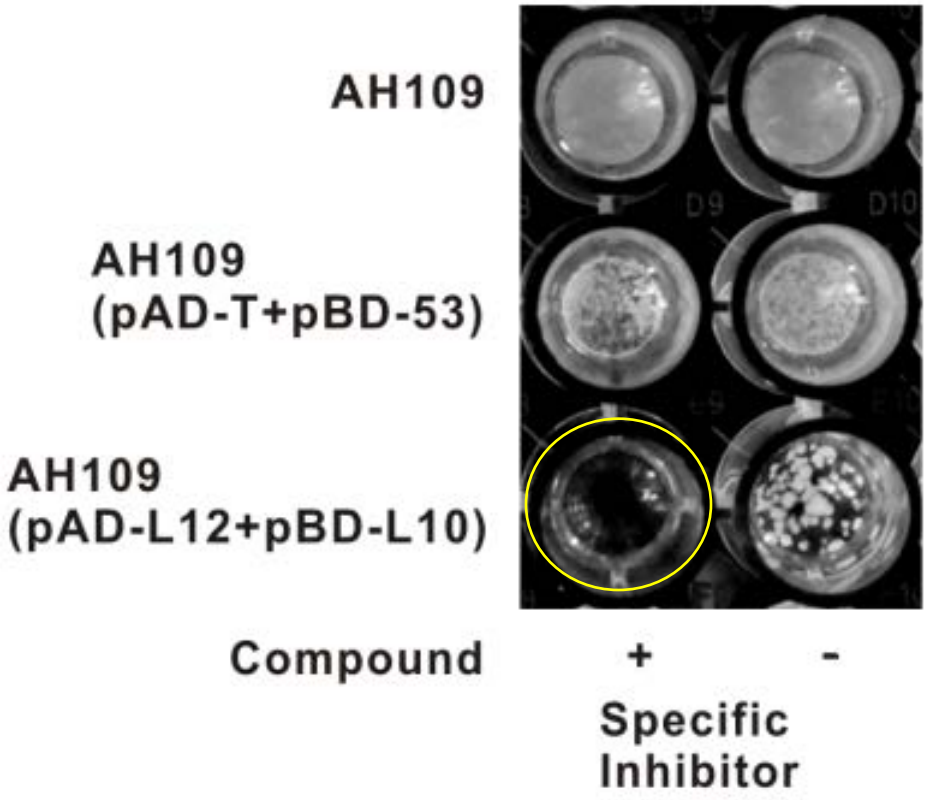


His^{-} Ade^{-} LacZ color change

C

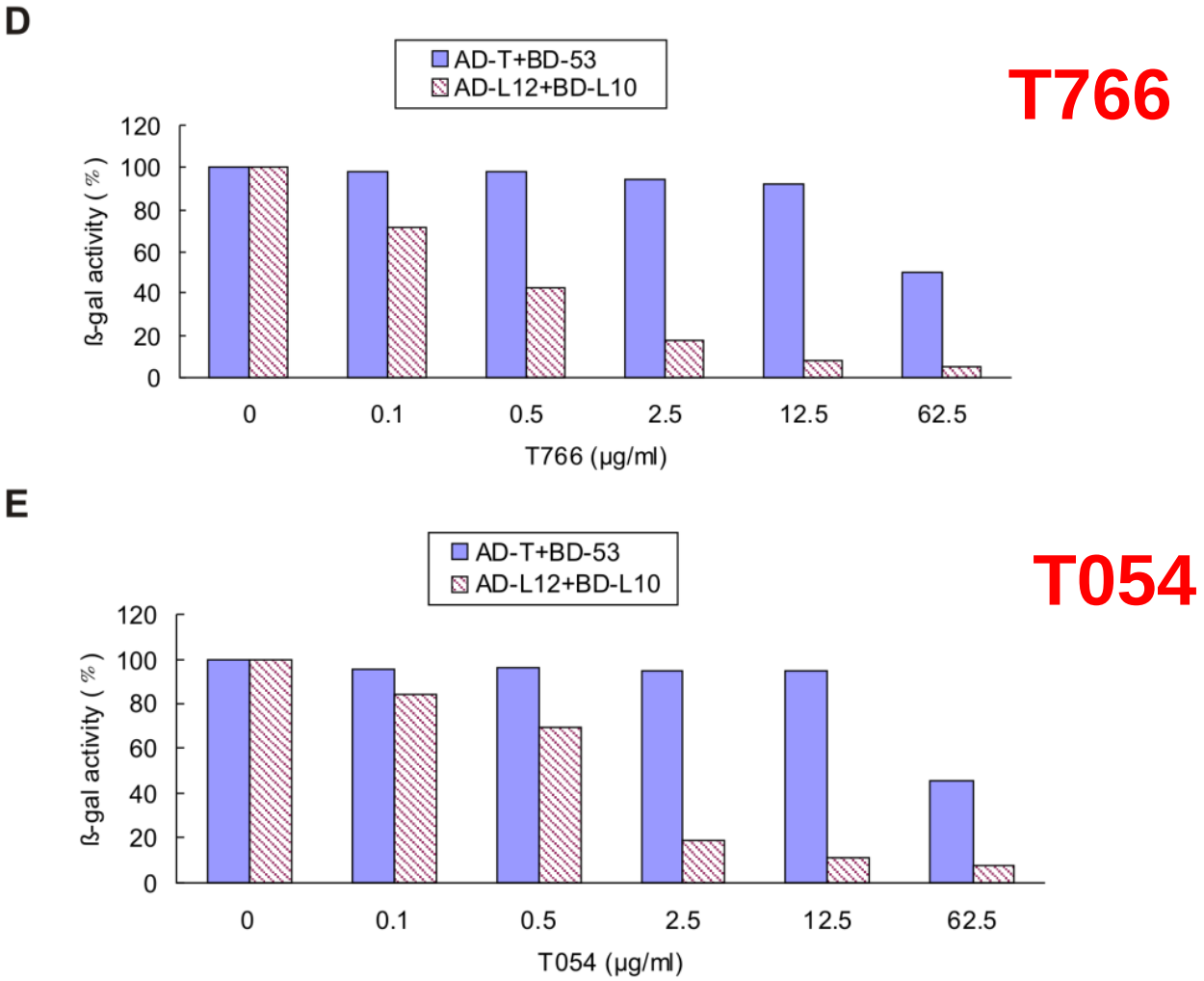


Compound library screen

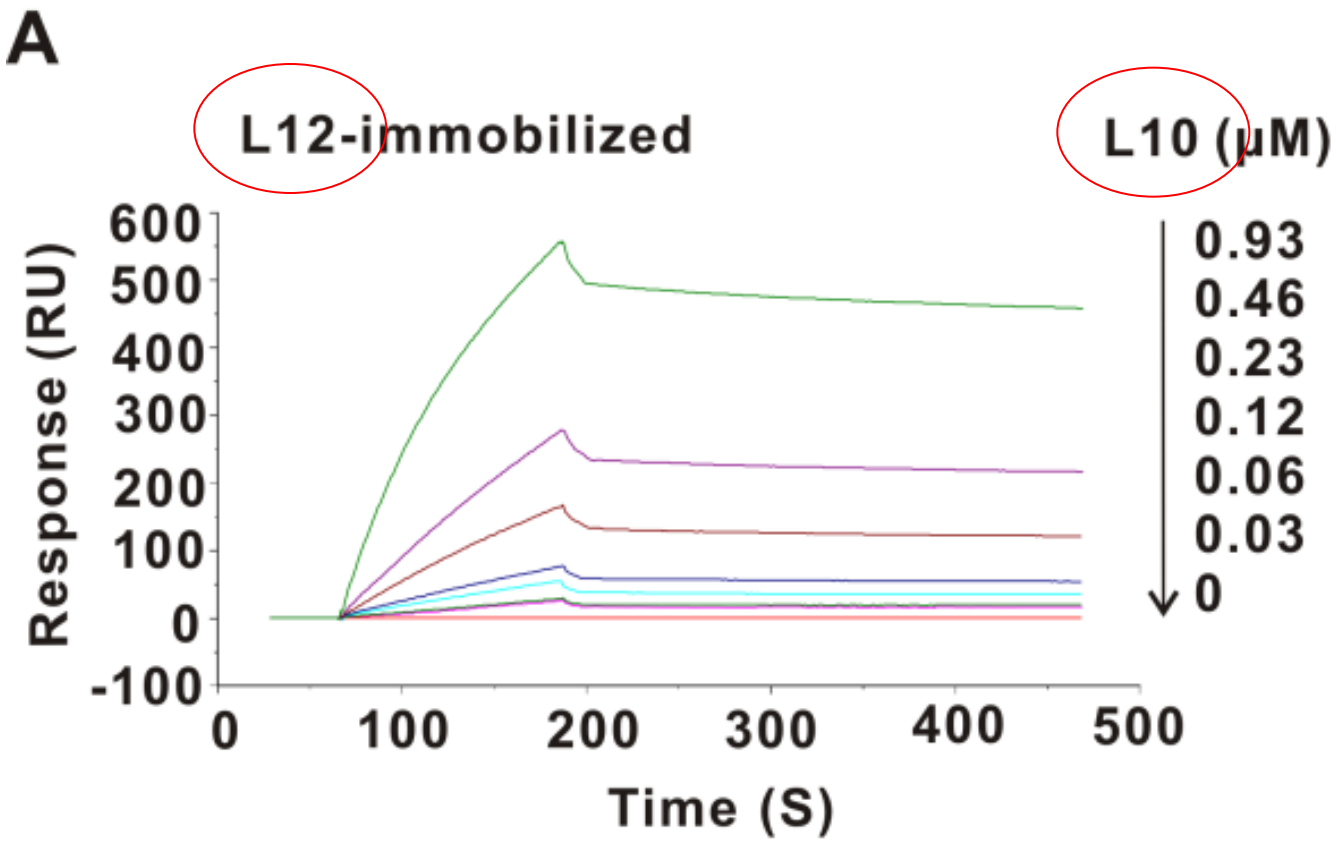


A compound inhibits the growth of yeast cells in Ade- His- medium

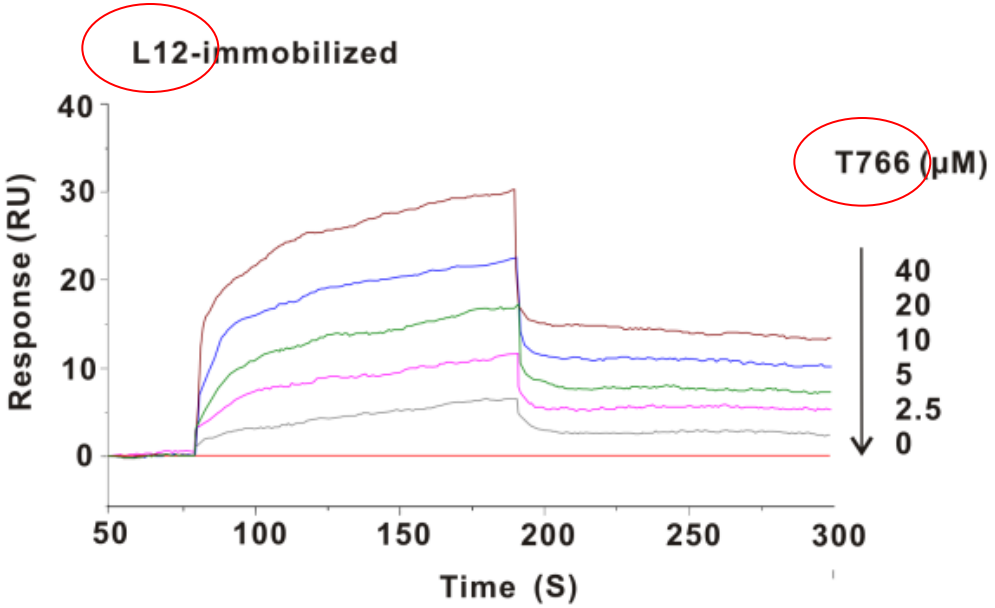
T766 and T054 inhibit the color change



Surface Plasmon Resonance (SPR)
L12 interacts with L10



T766 interacts with L12 but not L10



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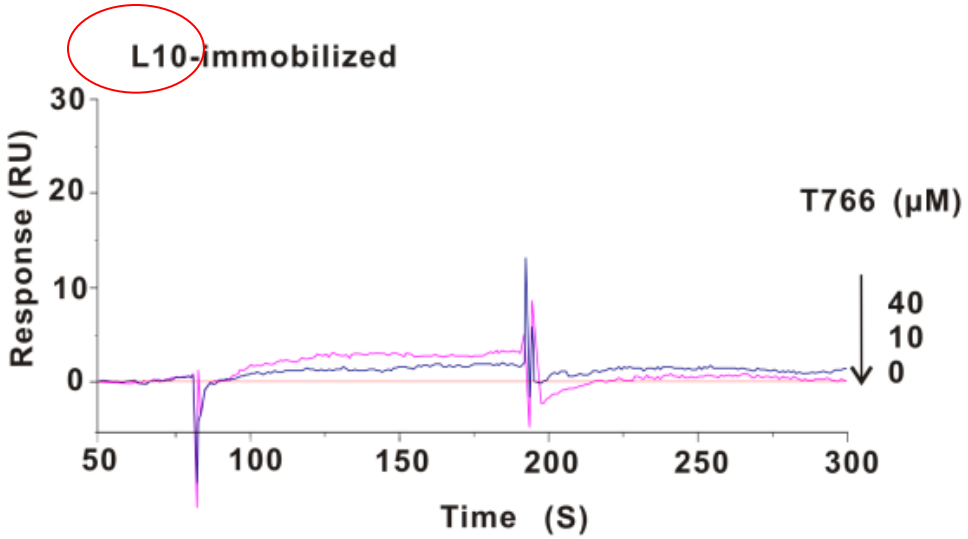
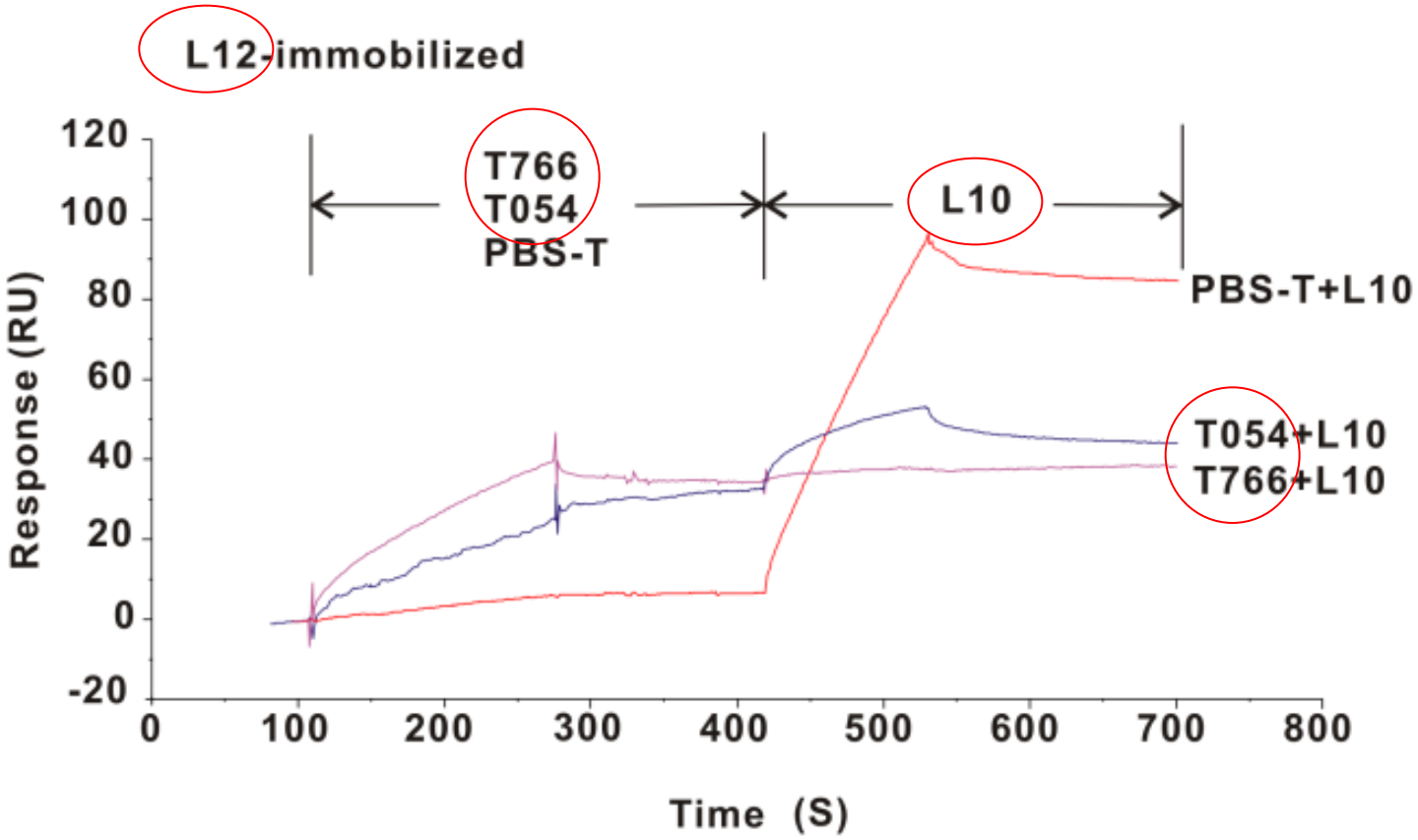
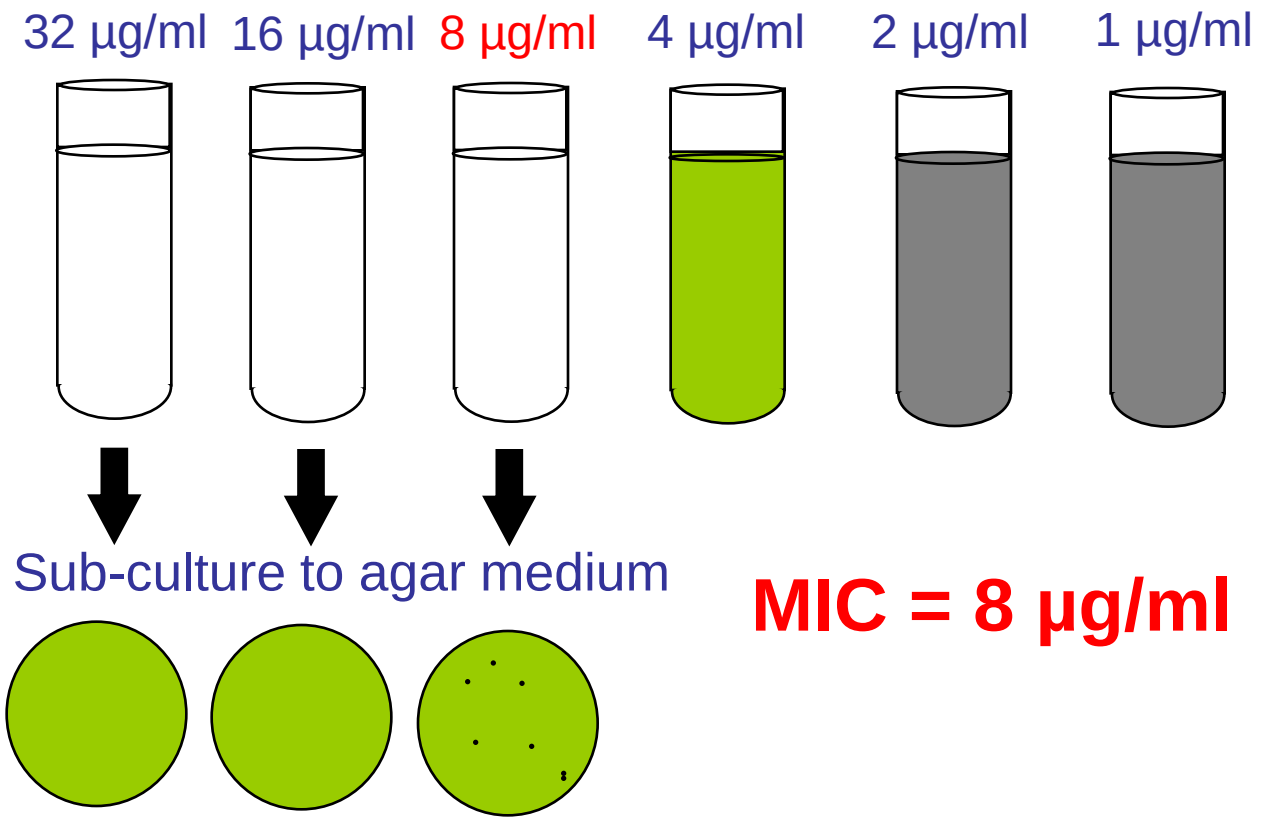


T766 and T054 disrupt L12-L10 interaction



Do the identified compounds inhibit TB growth?

Minimal Inhibitory Concentration (MIC)



MIC = 8 µg/ml

Yes, T766 and T054 can kill TB

The MICs of T766 and T054 against various *M. tuberculosis* strains

Compound	MIC (μg/ml)			
	H37Rv	STB-960	MDR-699	XDR-83
T766	0.312	0.25	4	64
T054	1.25	1.0	8	32
Isoniazid	0.156	0.125	64	8
Rifampin	0.156	0.125	64	64
Ethambutol	1.25	-	-	-

STB, clinical sensitive strain of *M. tuberculosis*; MDR, multidrug resistance; XDR, extensively drug resistance. Isoniazid and Rifampin were used as reference drugs. STB-960, MDR-699 and XDR-83 strains were all clinical isolates.

Summary

1. Established a system to detect L12-l10 interaction (yeast two-hybrid)

2. Isolated T766 and T054 (high throughput screen)

3. T766 and T054 disrupt L12-L10 interaction

4. T766 and T054 kill TB, but not other bacteria

5. T766 and T054 inhibit ribosome-mediated protein synthesis

6. T766 exhibit low toxicity to mice (LD50 = 650mg/ml)

7. Further analysis……..

Department of Biomedical Sciences

Alzheimer's disease

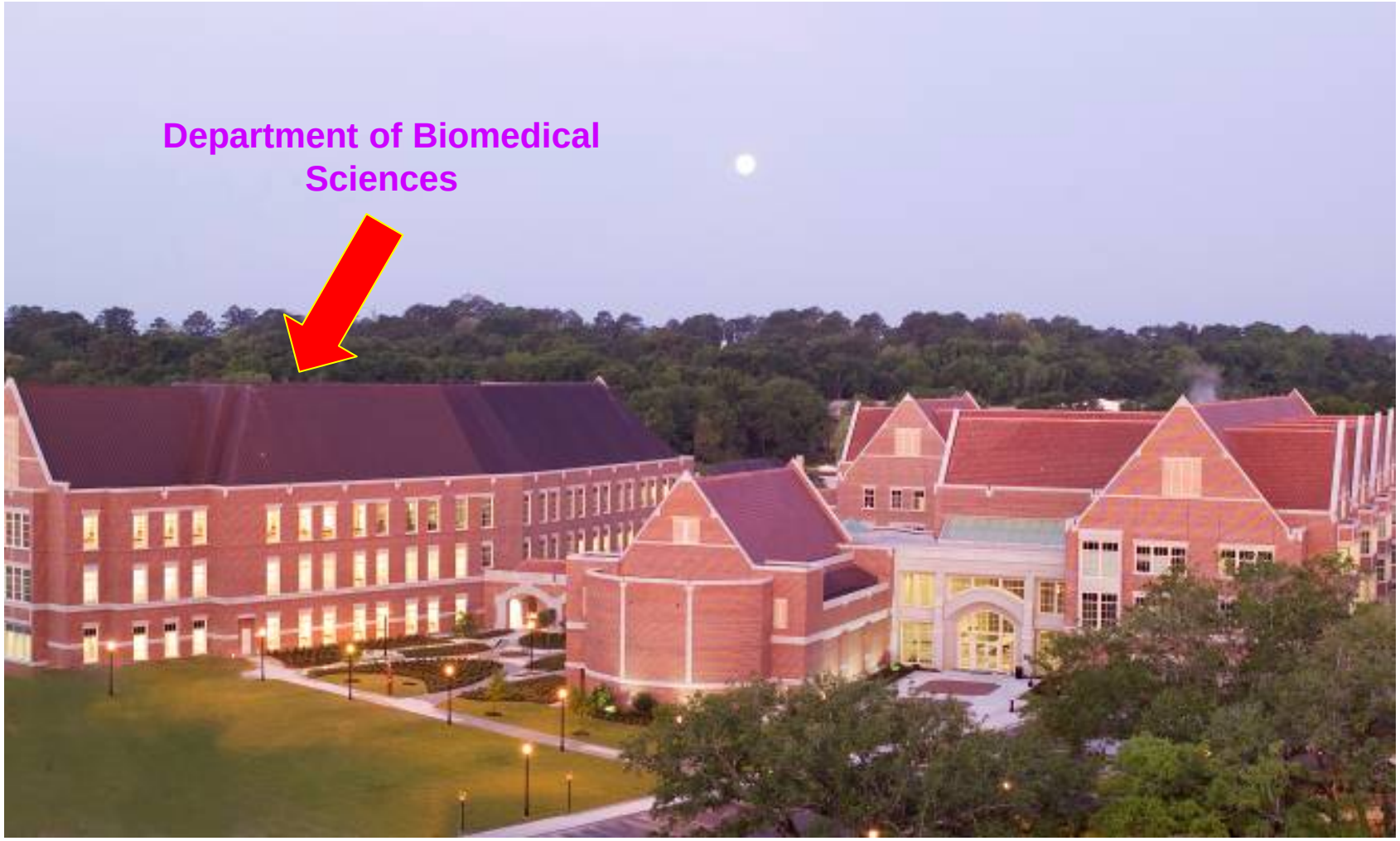
ADHD

Obesity

Sleep disorder

Others……

College of Medicine
Florida State University



Acknowledgement:

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