

Indo-Dutch WORKSHOP on Infectious diseases

Jan 21-23, 2010

DNA topoisomerases

DNA gyrase

DNA topoisomerase I

Other protein which influence topology



Regulation of gene expression

RNA polymerase

Transcription terminator Rho

Transcription elongation factors

(Nus factors, Mfd, Gre etc..)



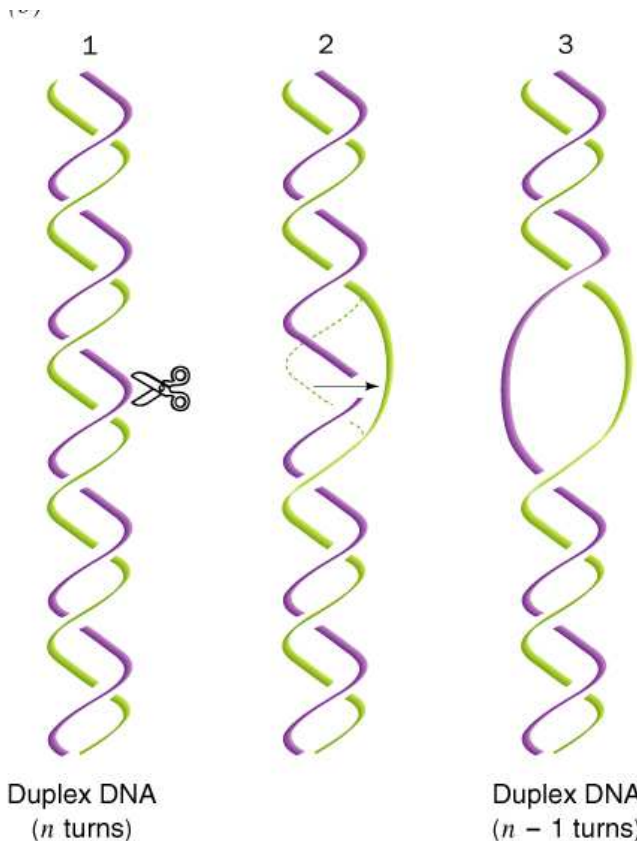
V. Nagaraja
IISc

Topoisomerases: Enzymes that Maintain Supercoiling of the DNA

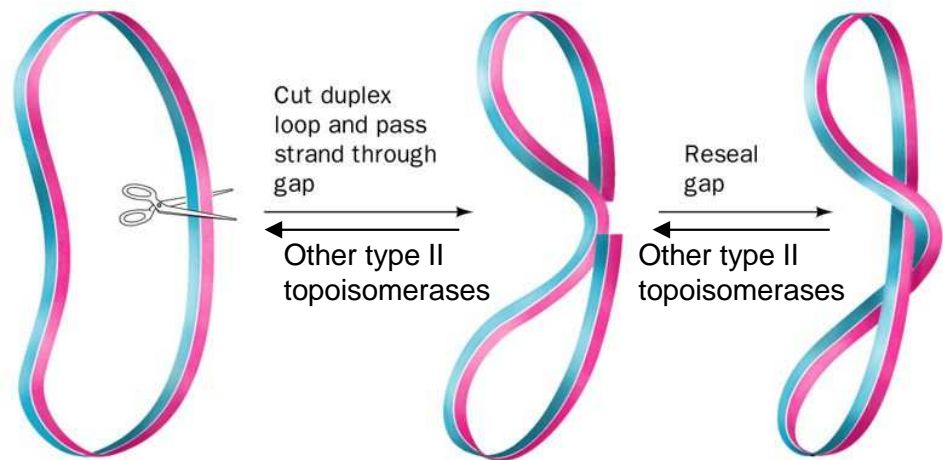
Two Types:

- Type I: Single strand break
- Type II: Double strand break

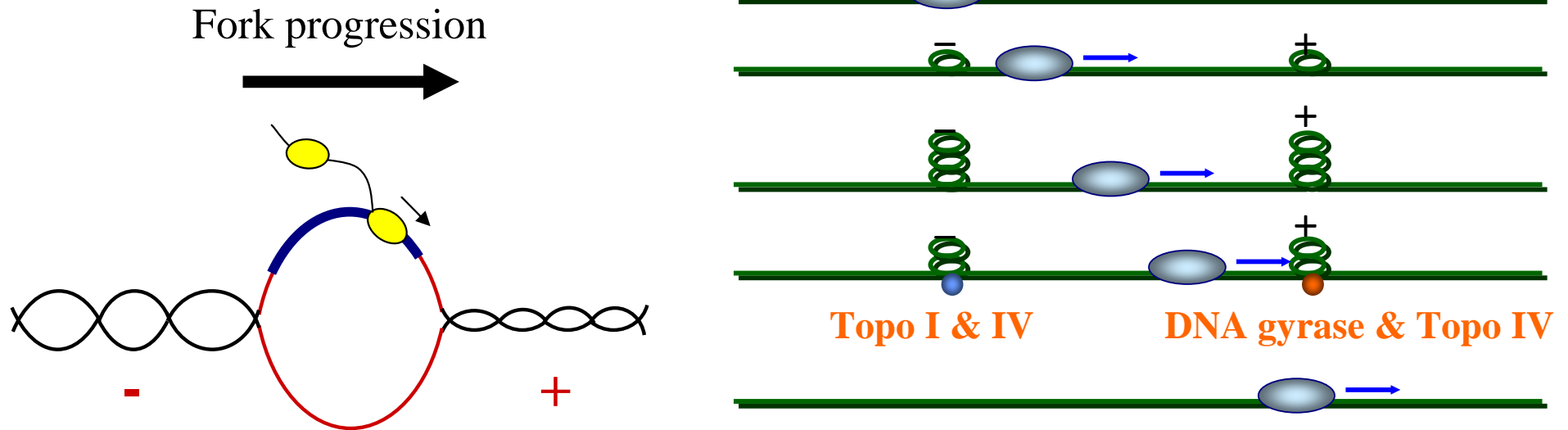
Type I Reaction



Type II Reaction



The strain of tracking: interplay of different enzymes



- Mutation in DNA gyrase can be suppressed by reducing the activity of topoisomerase I. Overexpression of topo IV rescues topo I loss of activity.
- Indirect evidence for the model *in vivo* and *in vitro* by inhibiting one or the other class of enzymes leads to the accumulation of either negative or positive supercoils

Drug target:-

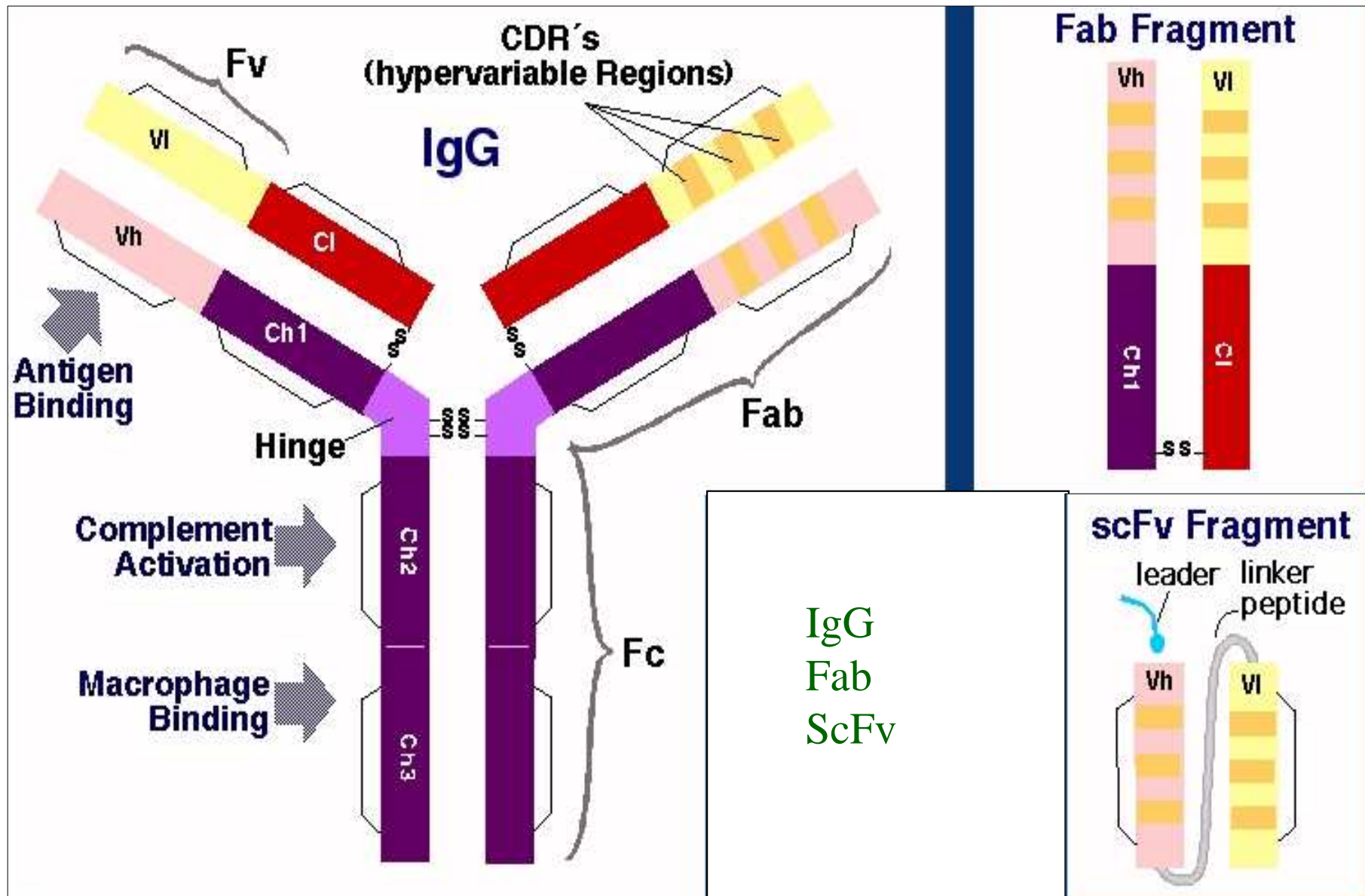
Developed peptide inhibitors against mycobacterial gyrase → their modification, intracellular delivery

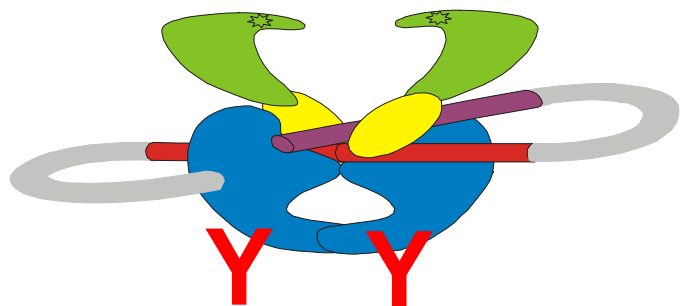
Topoisomerase 1 - unique enzyme- distinct from other eubacteria and higher organisms → development of specific inhibitors

Transcription termination factor Rho- structure/function studies; inhibitor development

DNA gyrase: RNAP physical interaction and functional dependence - topotranscriptosome → novel molecules to inhibit the interactions

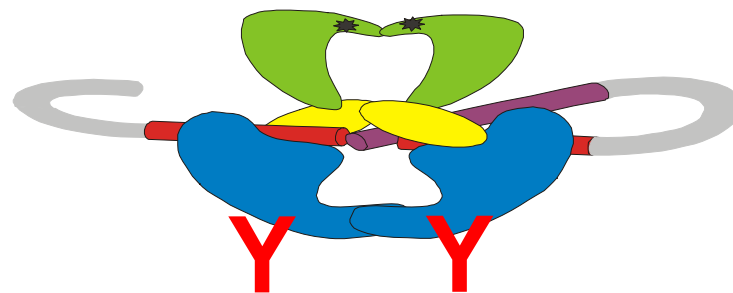
Engineering single chain antibody



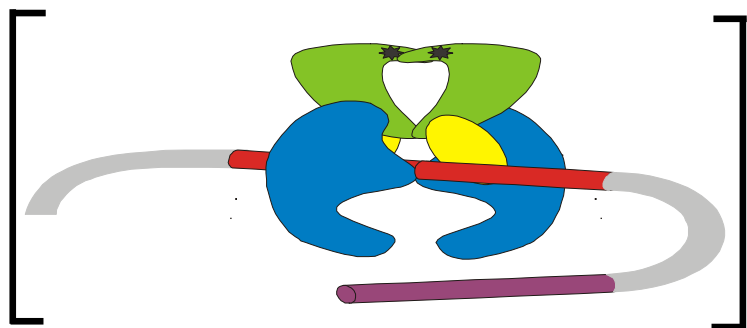


↑
IV

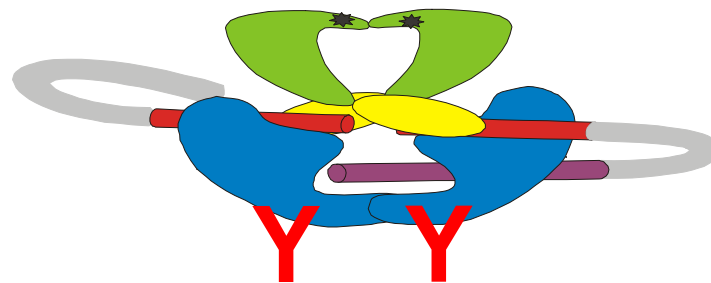
+ 2 ATP
→
I



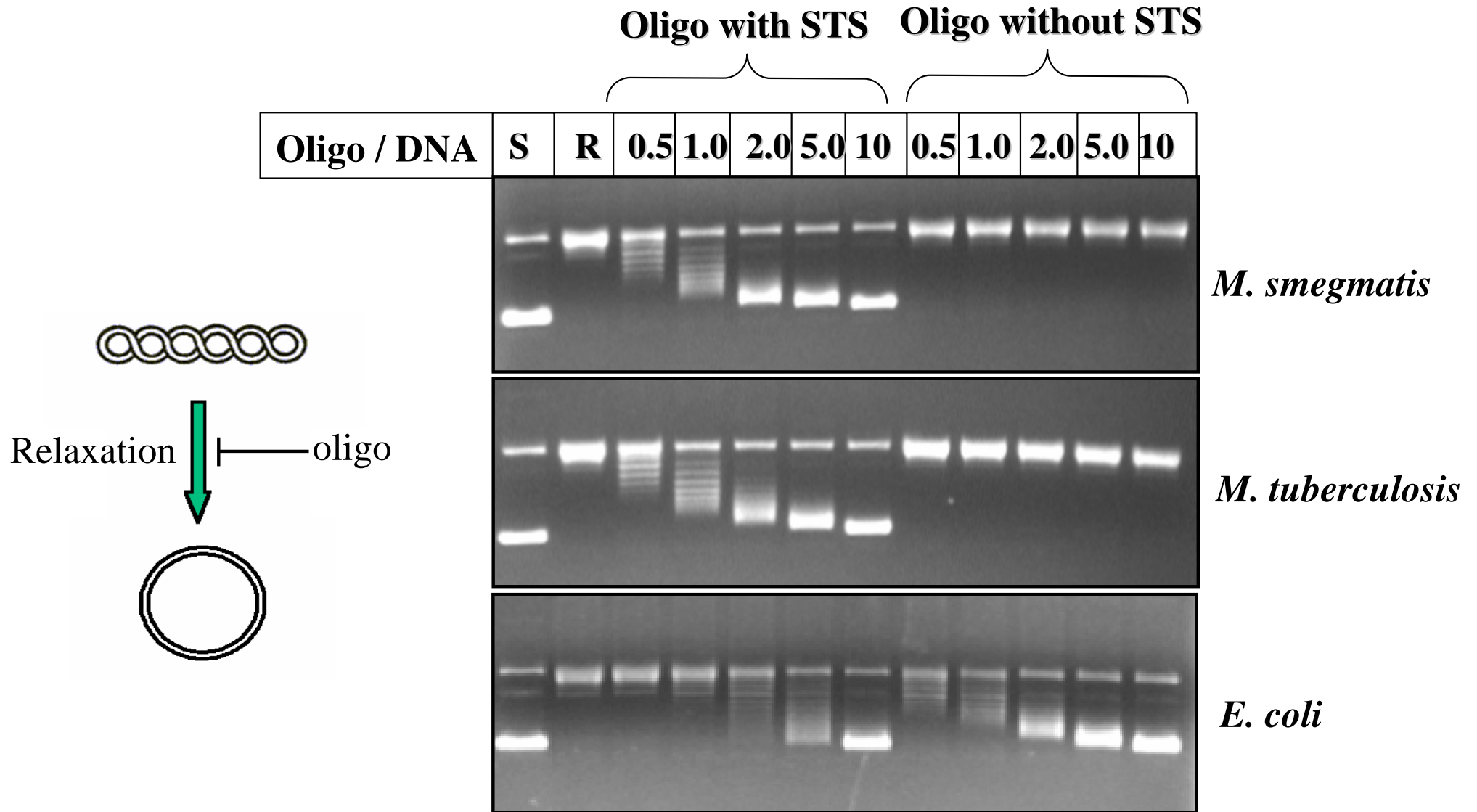
↓
II



←
~~III~~

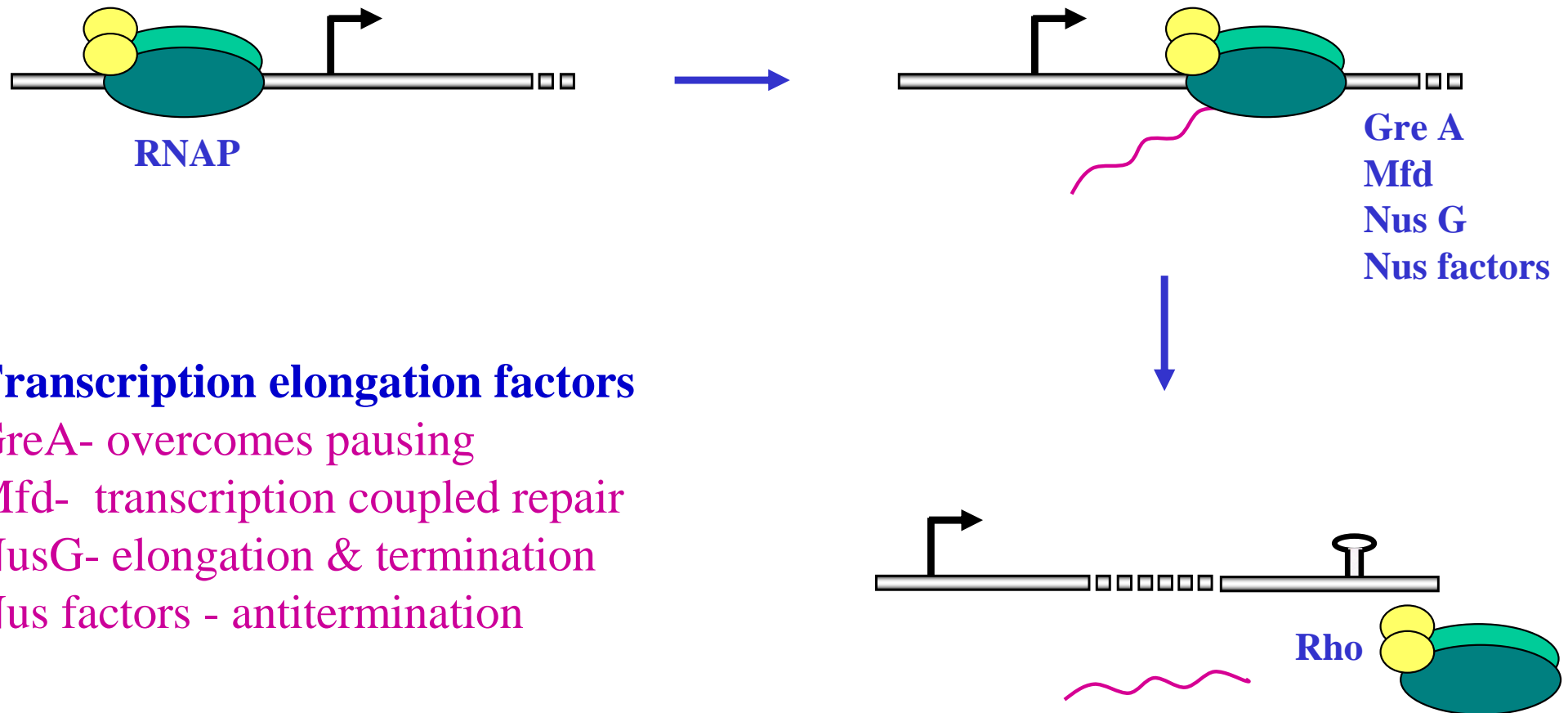


Inhibition of mycobacterial topoisomerase I relaxation activity by specific oligonucleotides (containing STS)



STS - Strong Topoisomerase I Site , S - Supercoiled DNA, R- Relaxation by topoisomerase I

GreA, Mfd and Nus factors in transcription elongation



Transcription elongation factors

GreA- overcomes pausing

Mfd- transcription coupled repair

NusG- elongation & termination

Nus factors - antitermination

Cloning, expression and interaction studies

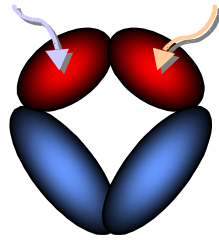
Modeling and structural analysis

Naturally occurring inhibitors of DNA gyrase

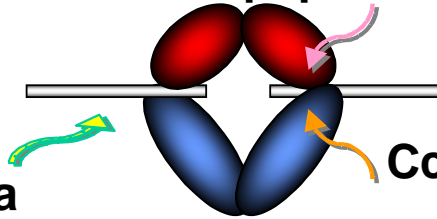
Interfere with ATPase activity

Stabilise gyrase-DNA covalent intermediate

Coumarins & cyclothialidines -
compounds from *Streptomyces sp.*



Microcin B17 - 43 amino
acid peptide from *pMccB17* plasmid



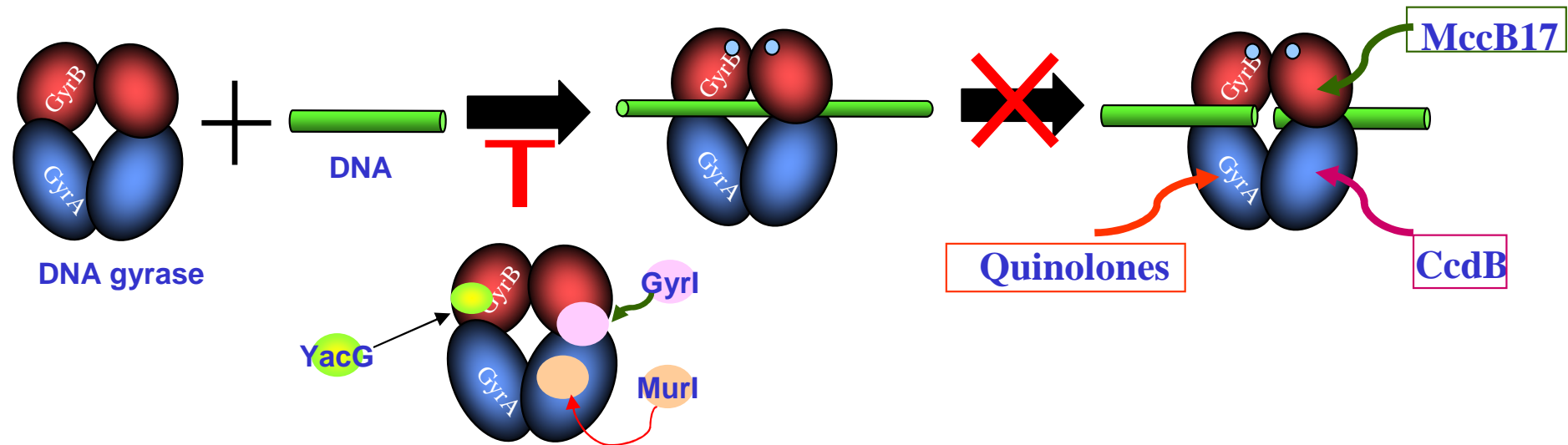
ParE- 12 kDa
toxin from RK2
plasmid

CcdB - 11.7 kDa toxin
from F plasmid

Chromosomally encoded inhibitors

- ♣ Gyrl from *E. coli*
- ♣ Thioredoxin [TrxC] from *R. capsulatus*
- ♣ MfpA from *M. smegmatis*
- ♣ Glutamate racemase [Murl] from *E. coli*, *B. subtilis* and *M. tuberculosis*

Model for action of Endogenous inhibitors on DNA gyrase



Function of topoisomerase modulators in the cell

Cell survival
 Protection against external agents (poisons and other DNA gyrase inhibitors)

GyI, YacG, Murl

MfpA from *Mycobacterium sp.*

Transient modulation of the enzyme activity

Eg. oxidized TrxC inhibits in *Rhodobacter capsulatus*

Reduced TrxA stimulates in *Rhodobacter sphaeroides*

Murl? YacG?

Sequestration of the enzyme

- Away from the site of action
- To the site of action

Murl ?
 YacG?



Thank You