The Central Dogma: DNA, RNA, and Protein

Overview

Essential processes for copying and interpreting biological information:

1. REPLICATION – the synthesis of a new DNA molecule from an existing template

2. TRANSCRIPTION – synthesis of an RNA molecule using a DNA template

3. TRANSLATION – synthesis of protein using an RNA template

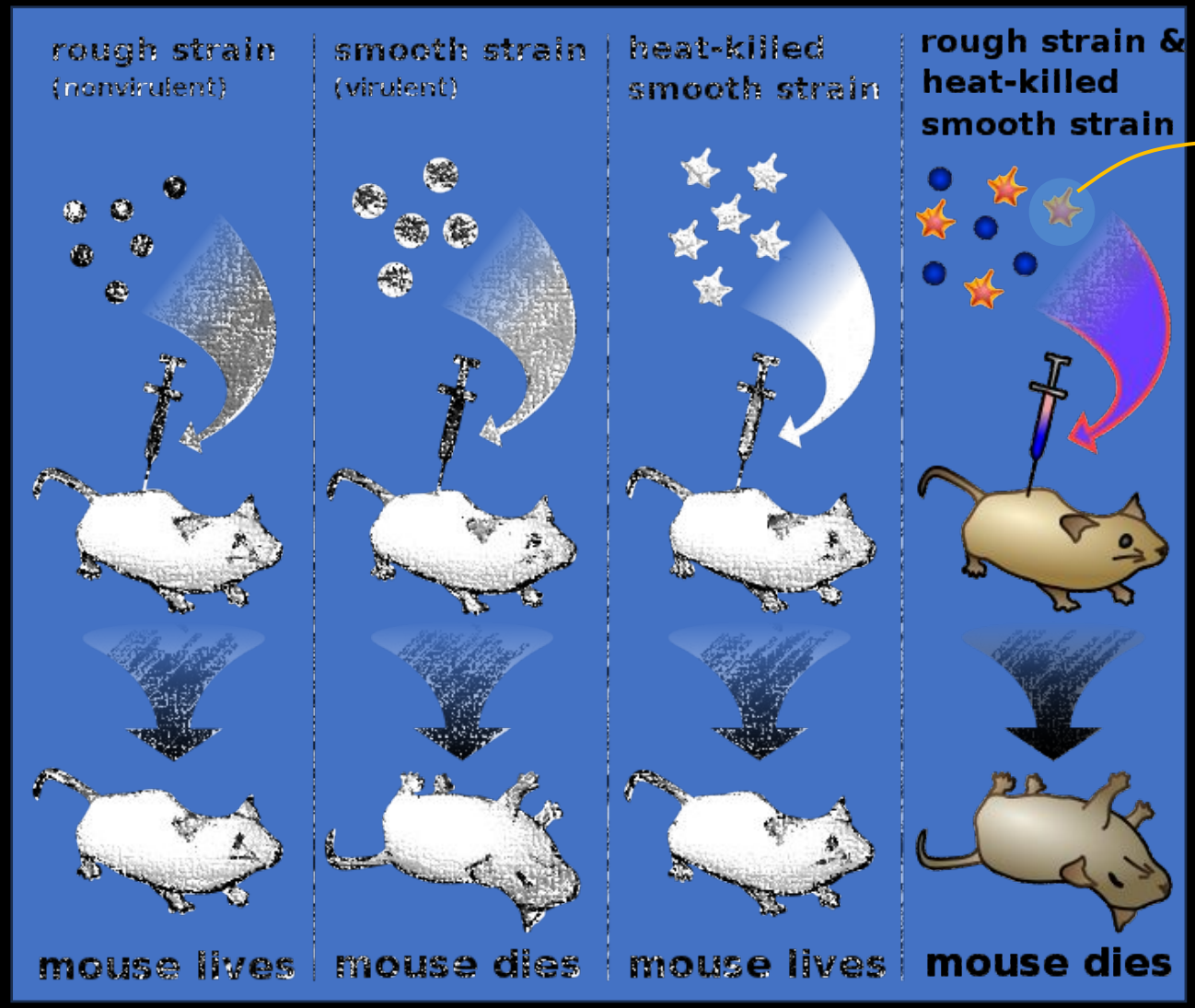
Griffith's experiment – something's moving between organisms

Streptococcus

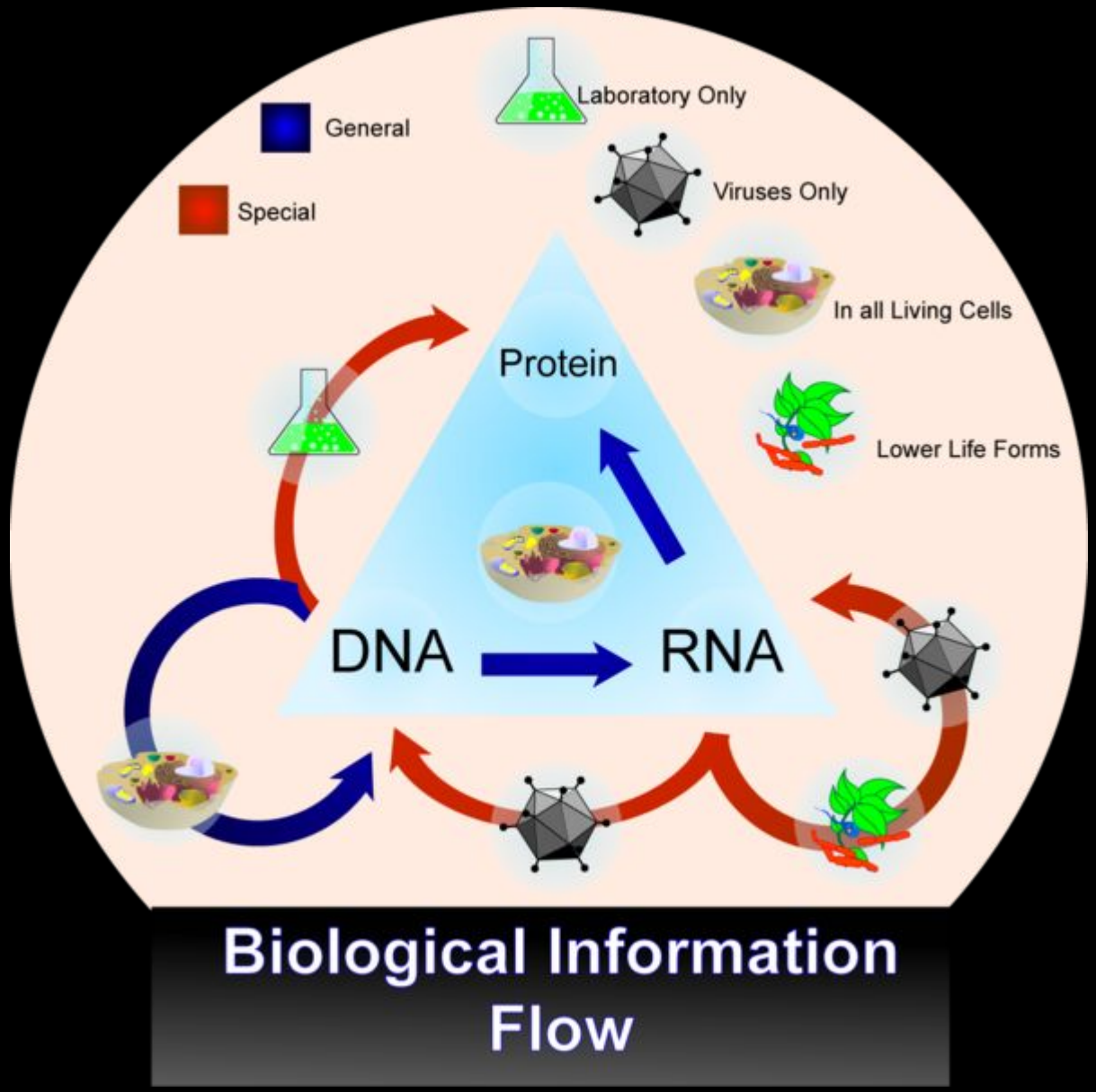
Frederick Griffith (1928) *J Hygiene*

Image: Madeleine Price Ball, Wikimedia

Avery-MacLeod-McCarty experiment – that something is DNA

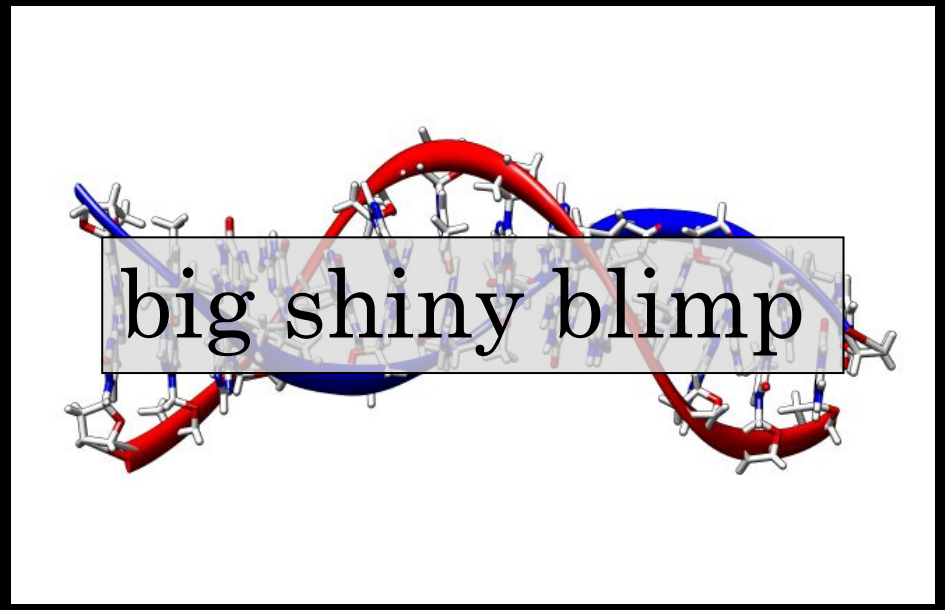


Avery et al. (1944) *J Exp Med*



The 'Central Dogma' – From Information to Function

Mike Jones - http://commons.wikimedia.org/wiki/File:CDMB2.png



Replication

big shiny blimp
big shiny blimp
big shiny blimp
big shiny blimp
big shiny blimp

Transcription

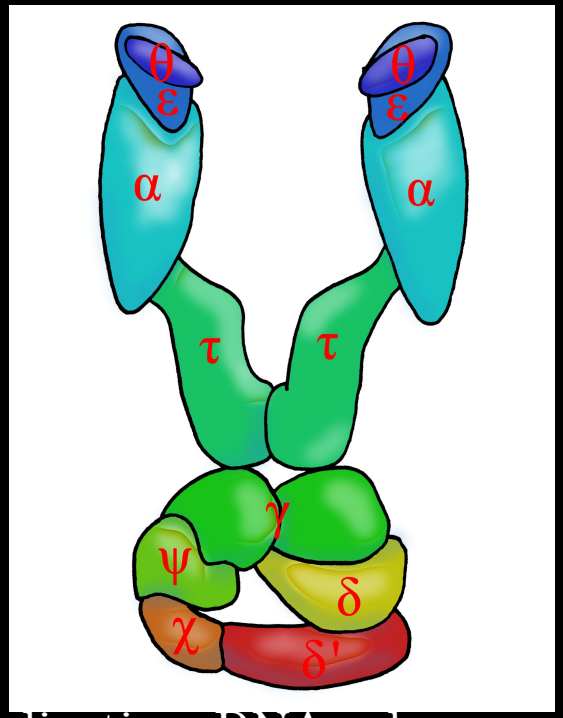
BIG SHINY BLIMP

Translation

-... .. --. ... . -. -.-- -... .-.. .. -- .--.

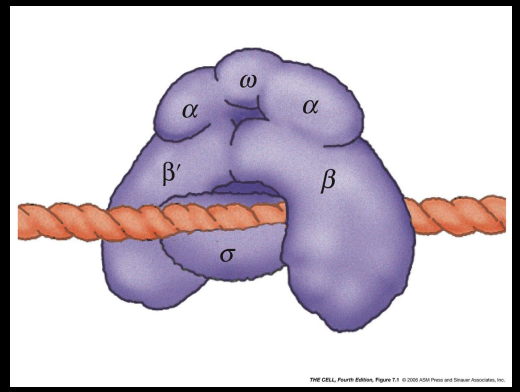
Key steps and commonalities

All three processes are carried out by **multi-protein complexes** (sometimes with extra bits thrown in)

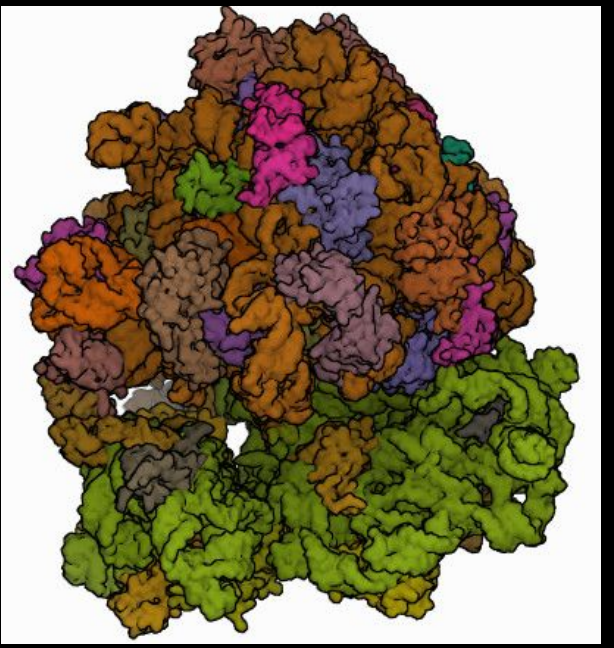


Replication: DNA polymerase

https://en.wikipedia.org/wiki/DNA_polymerase_III_holoenzyme



Transcription:
RNA polymerase

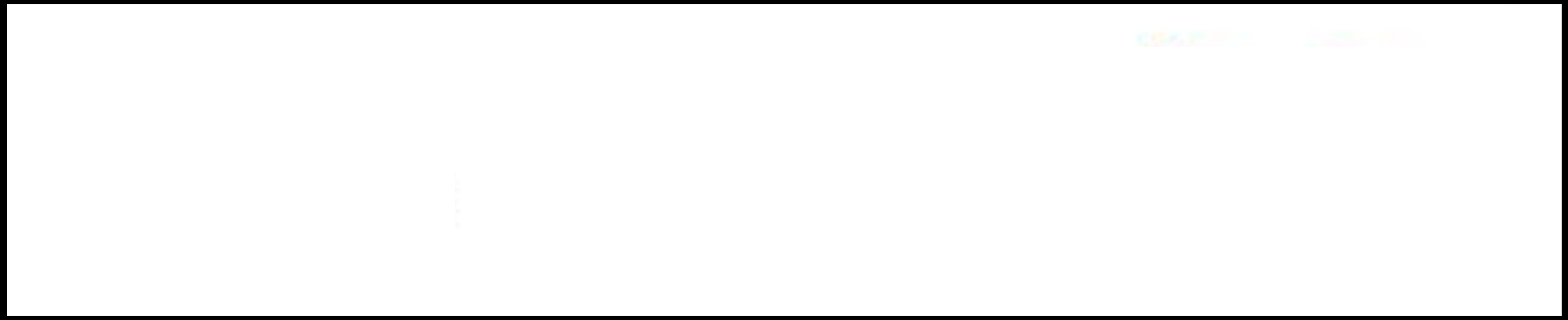


Translation:
Ribosome

https://www.rcsb.org/structure/5V93

Key steps and commonalities

All processes and phases are **regulated** and have three phases:



Initiation Elongation Termination

https://www.pnas.org/content/98/1/5/tab-figures-data

Key steps and commonalities

Processes in **eukaryotes** tend to be more complex than those in **prokaryotes**

>

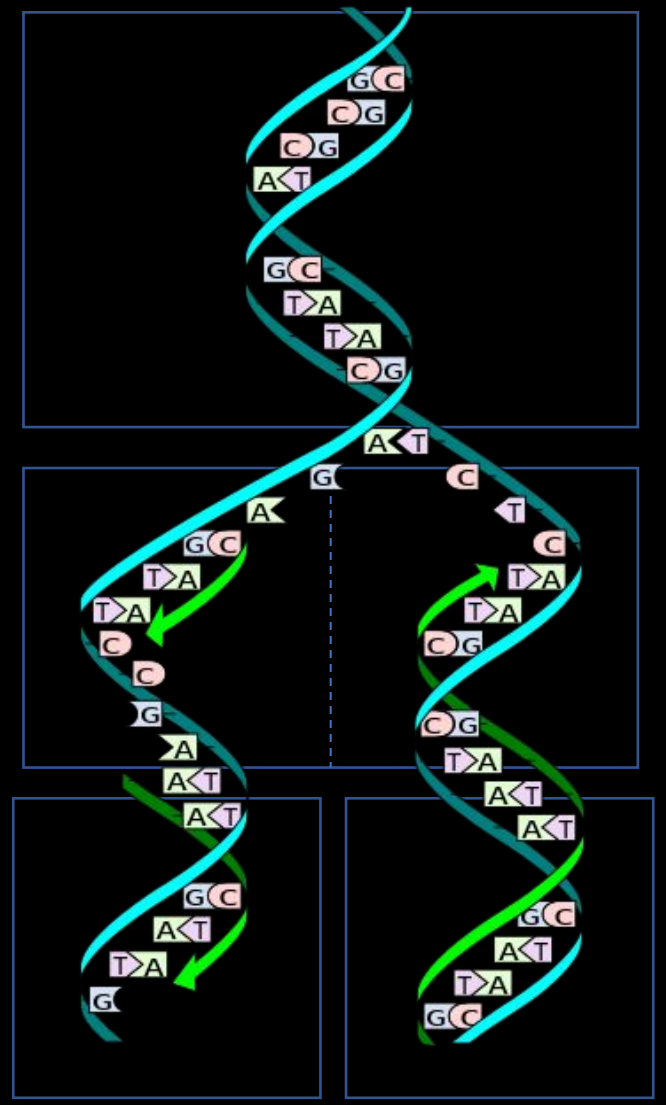
REPLICATION:
from DNA to more DNA

The replication process

(1) DNA is UNWOUND

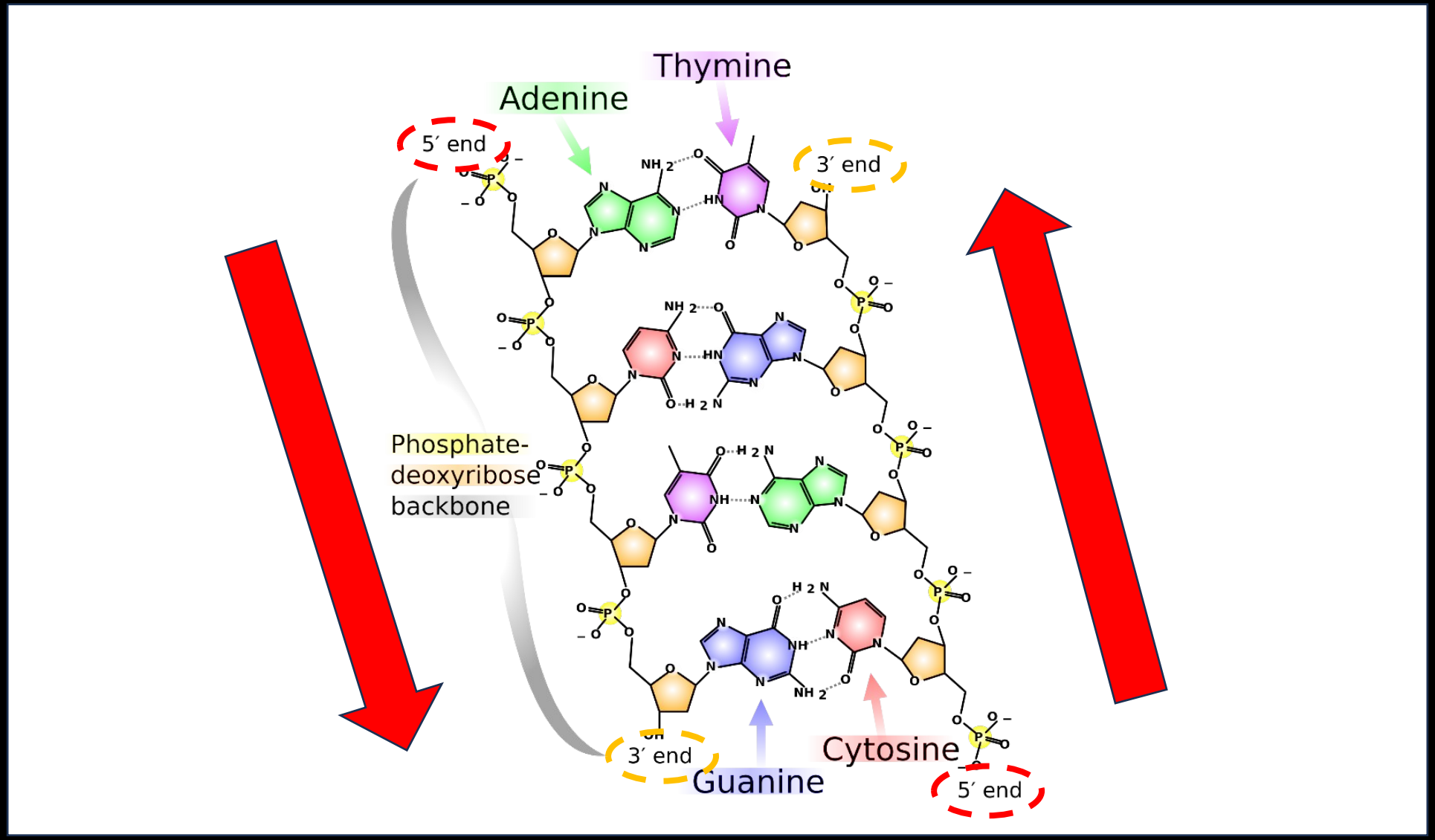
(2) A copy of EACH STRAND is made independently

(3) Each copy is packaged into a cell



http://en.wikipedia.org/wiki/File:DNA_replication_split.svg

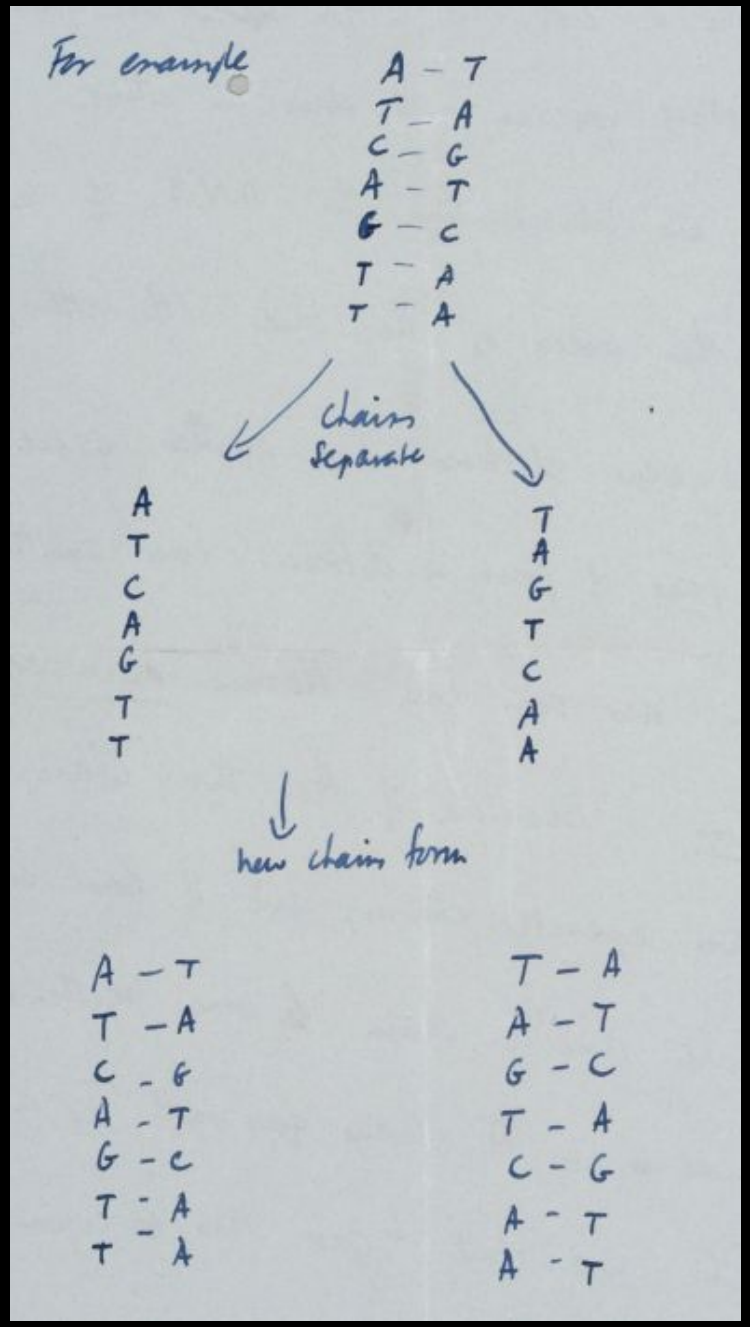
DNA has polarity



http://en.wikipedia.org/wiki/File:DNA_replication_split.svg

You can now see how Nature makes copies of the genes. Because if the two chain unwind into two seperate chains, and if each chain then makes another chain to come together on it, then because A always goes with T, and G with C, we shall get two copies where we had one before.

From Francis Crick's letter to his son Michael, 1953
$5.3M at auction

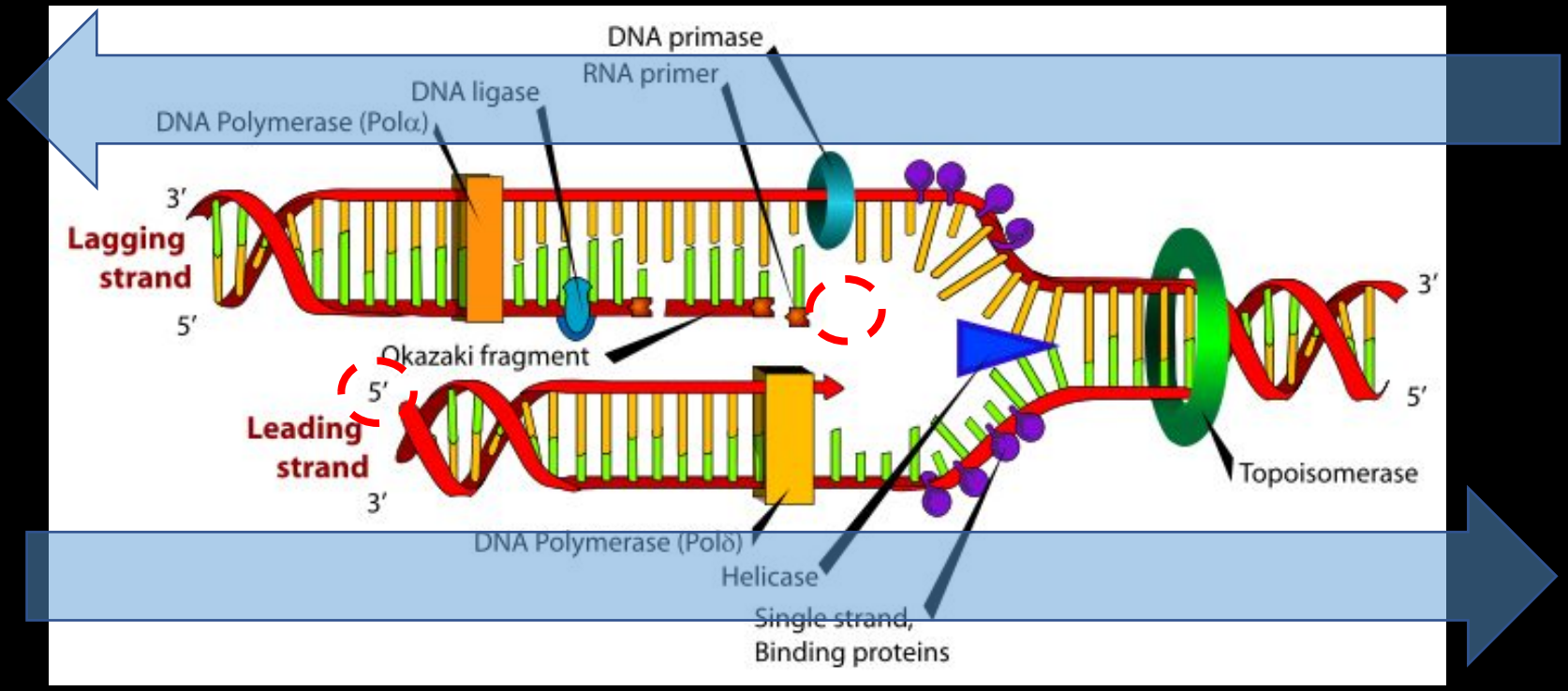


http://voices.nationalgeographic.com/2013/04/11/francis-cricks-letter-to-son-describing-dna-auctioned/

Replication proceeds in the 5′ – 3′ direction

But DNA is antiparallel (the two strands point in opposite directions)!

So replication proceeds *differently* on each strand (leading strand is *way* easier)



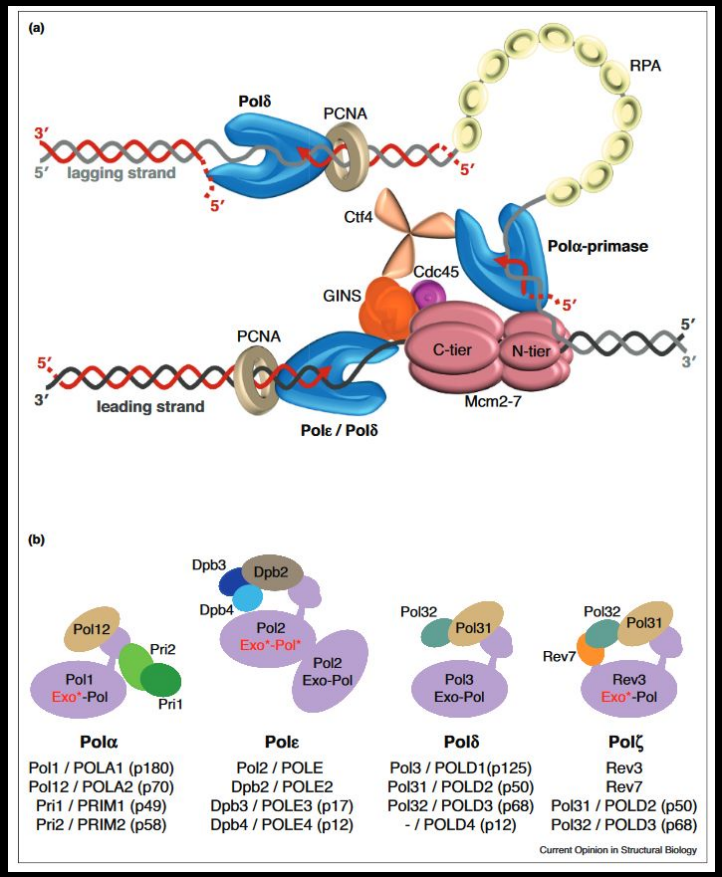
http://en.wikipedia.org/wiki/File:DNA_replication_en.svg

DNA polymerase III (bacteria): >6 different subunit types

http://www2.mrc-lmb.cam.ac.uk/groups/mlamers/Site/Home.html

Eukaryotes:
At least 14 different DNA polymerase complexes

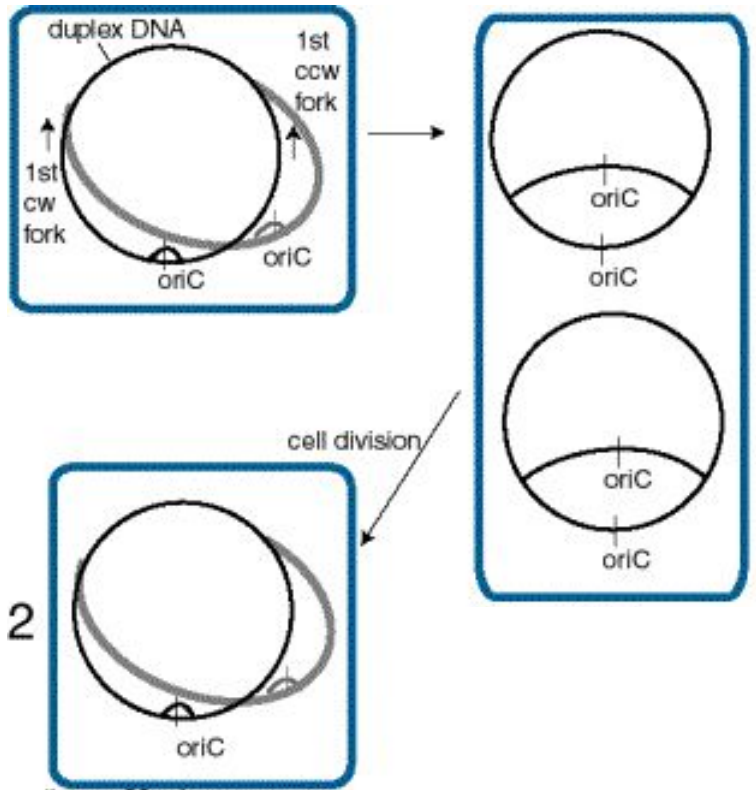
DNA polymerase	Function
α	DNA replication/priming
β	Base excision repair
γ	Mitochondrial DNA replication
δ	Chromosomal replication/excision repair
ε	Chromosomal replication/repair
ζ	REV3: error-prone bypass synthesis
η^*	RAD30: error-free bypass of UV-induced CPDs
θ	DNA repair
ι^*	RAD30B: bypass synthesis
κ^*	DinB: bypass synthesis
λ	Base excision repair
μ	Non-homologous end joining
σ	Sister chromatid cohesion
REV1*	Deoxycytidyl transferase



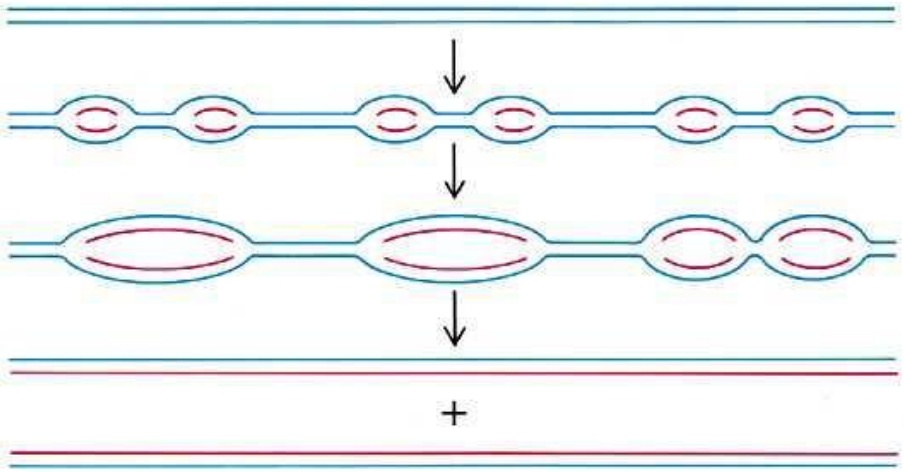
Carty et al. (2003) *Biochemical Society Transactions*

Jain et al. (2018) *Current Opinion in Structural*

Replication



E. coli: Single origin
~40 minutes



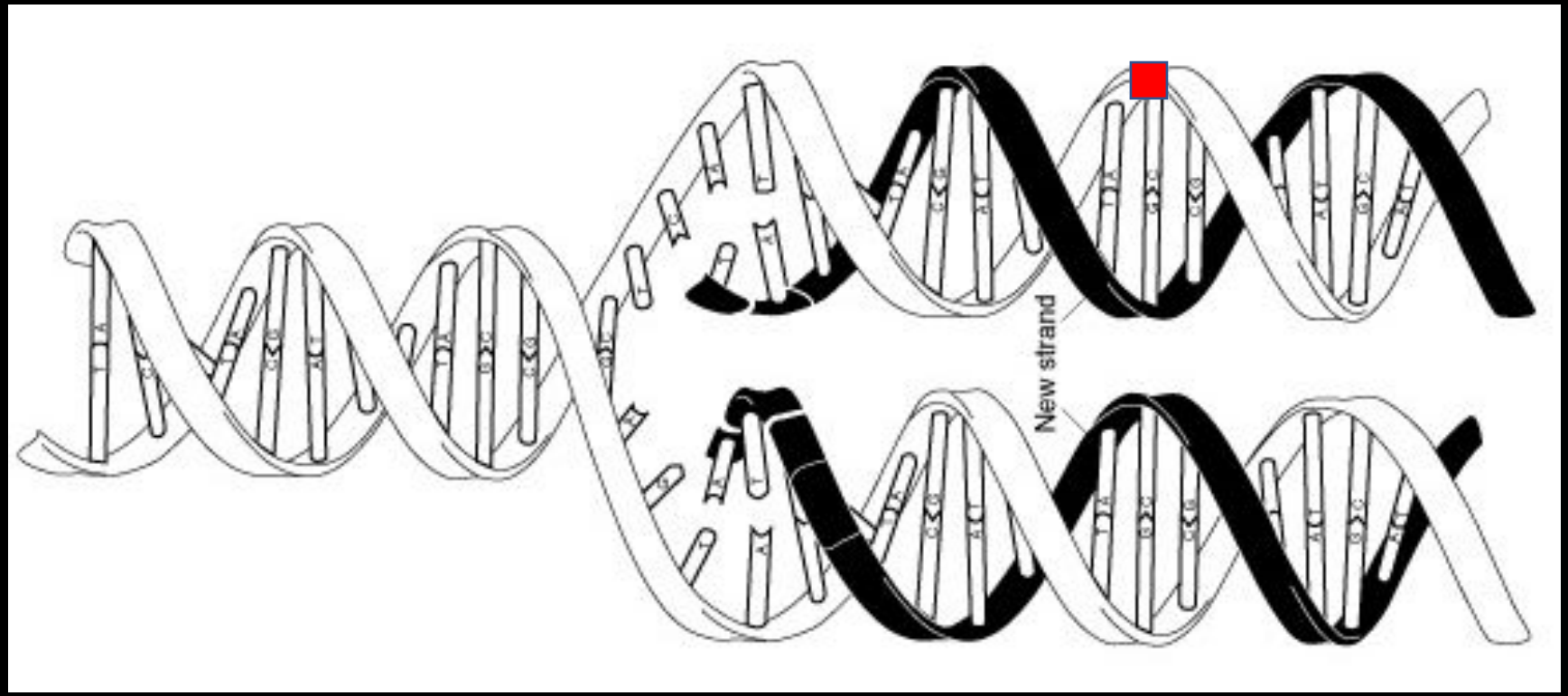
Humans: Multiple origins per chromosome
1+ Hours

http://www.personal.psu.edu/rch8/workmg/Replication2Ch6_files/image049.png
http://www.mun.ca/biochem/courses/3107/images/Stryer/Stryer_F37-13.jpg

Mutations

When DNA polymerase makes an error that is not caught by the 'proofreading' mechanism, a *mutation* results

G inserted where A is supposed to go (oops!)



Fidelity

~ 10^{-8} error rate (varies by species)

Without proofreading, error rate ~ 10^{-5}

Some viruses: 10^{-3}

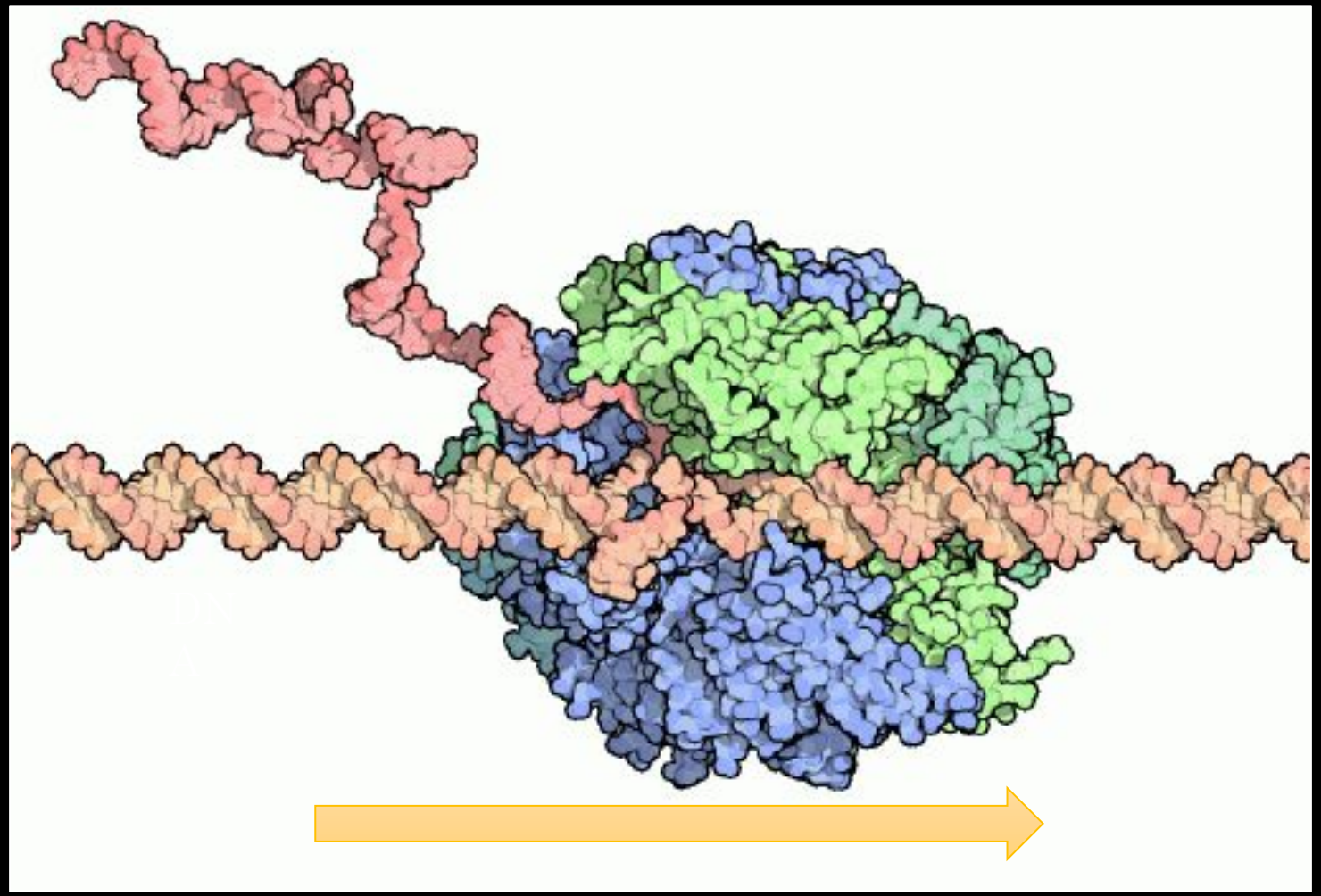
Mutation patterns are **not random**!

TRANSCRIPTION:
from DNA to RNA

What transcription looks like

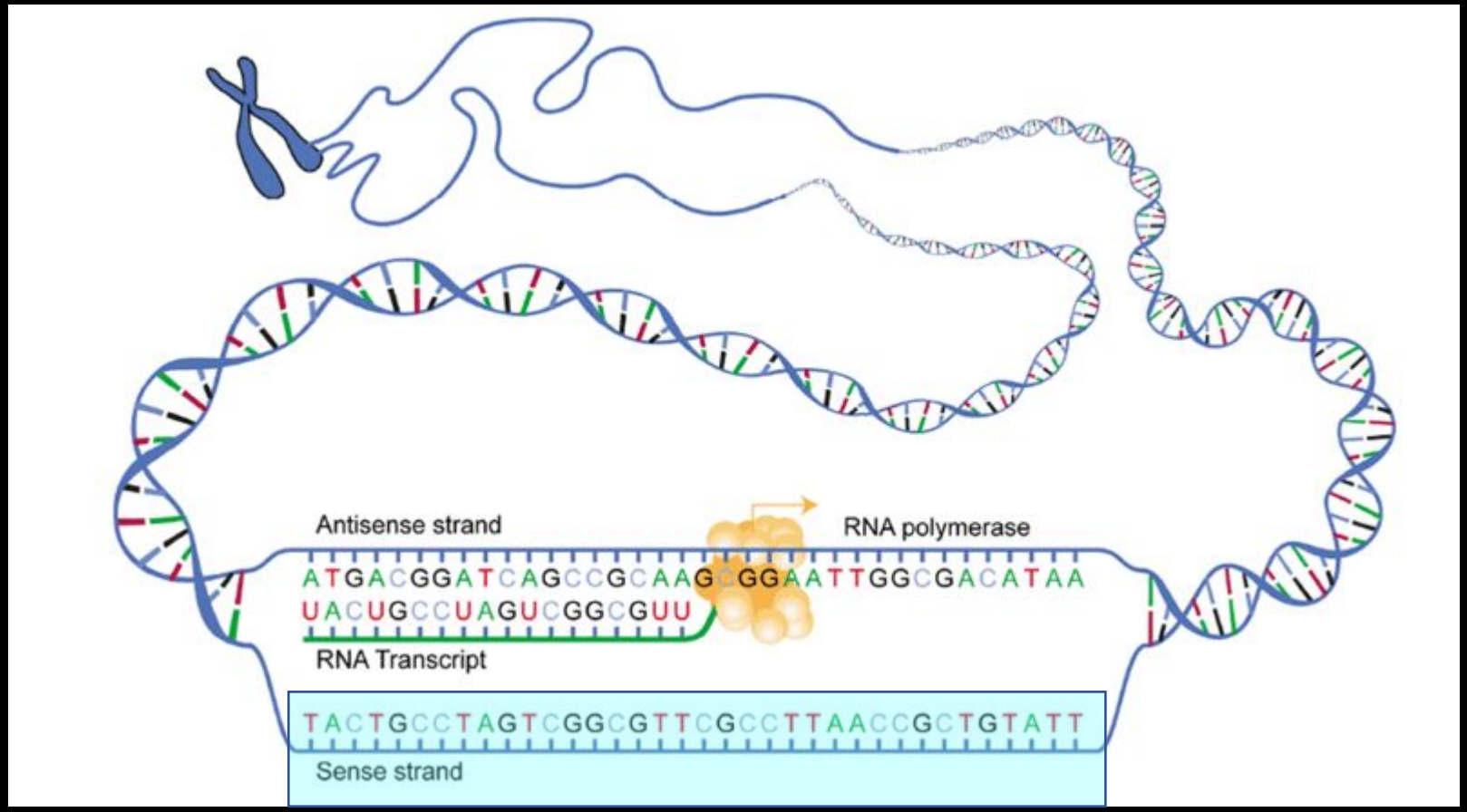
An RNA molecule is produced

The DNA template is read by RNA polymerase…



http://www.rcsb.org/pdb/101/motm.do?momID=40

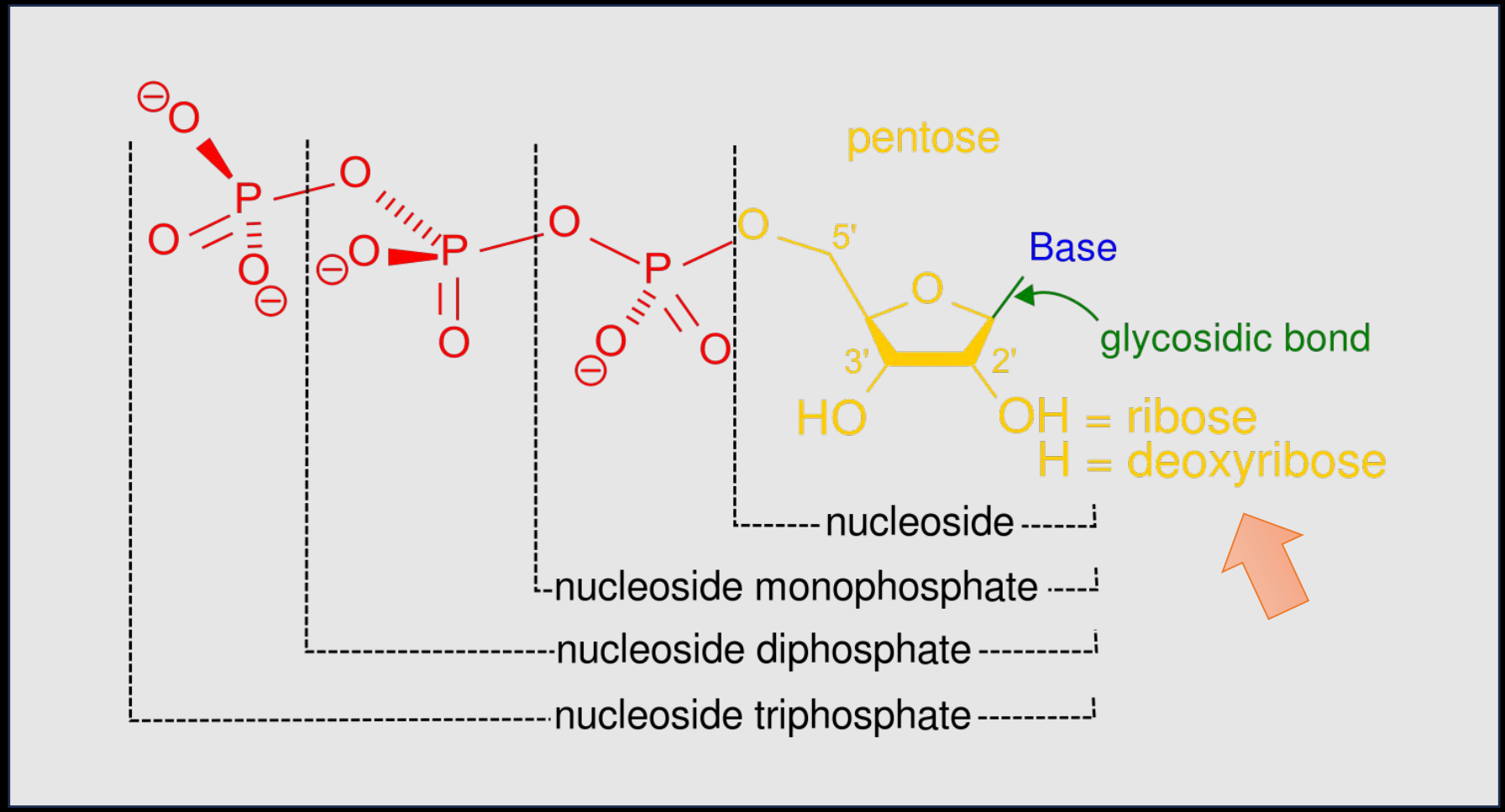
Transcription



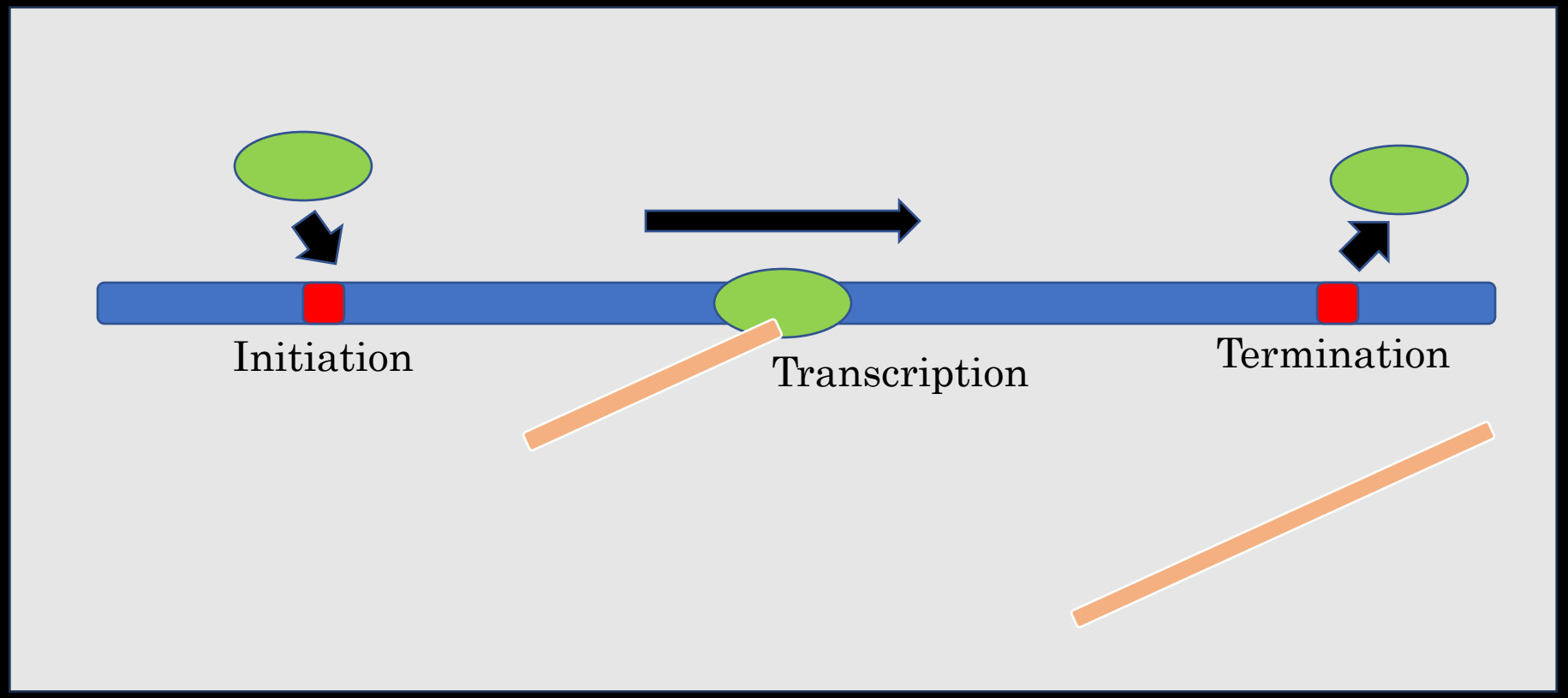
(This is the one we typically write out)

http://commons.wikimedia.org/wiki/File:DNA_transcription.svg

One silly oxygen, a whole world of difference



http://en.wikipedia.org/wiki/File:Nucleotides_1.svg

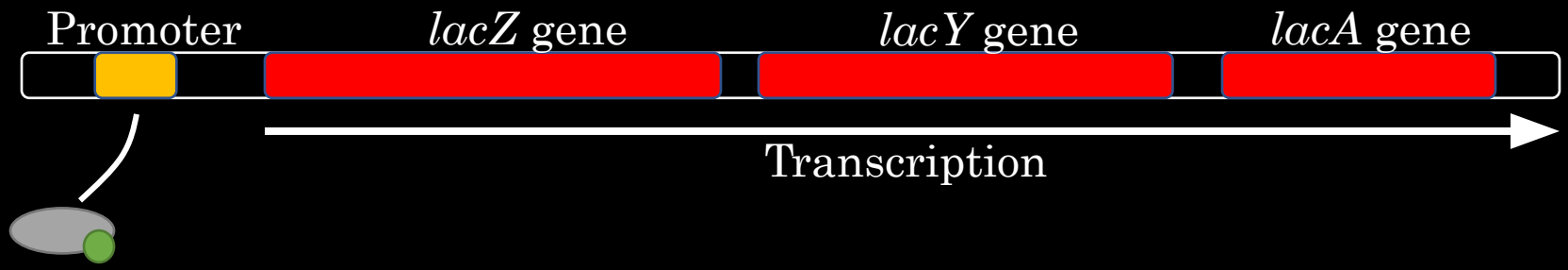

Initiation
Transcription
Termination

Regulating transcription: the promoter

lac operon in *E. coli* – breakdown of lactose sugar (simplified)

operon = *set of genes transcribed together but translated independently*

RNA polymerase must recognize a stretch of DNA upstream of the genes to be transcribed – the **promoter**



RNA polymerase with sigma factor

Sigma factor binding sites in front of different genes

```
tttatgttgctttttgtaaacagattaacacctcgtcaaaatcctgctattctgcccgtTgcggtactgggcatttaccc
atcgtggtaagacctgccgggatttagttgcaaatttttcaacatttatacactacgaaAaccatcgcgaaagcgagttt
gttcgtgaatttacaggcgttagatttacatacatttgtgaatgtatgtaccatagcacgAcgataatataaacgcagcaa
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agaaactaatattagacgtaaatattgaaatttttatatttttttcttatttaggctttgcAtttggcaaaattttgaggca
```

???

(more on this in the machine-learning section)

Extracted from RegulonDB (http://regulondb.ccg.unam.mx/)

The fate of transcripts

Coding RNA

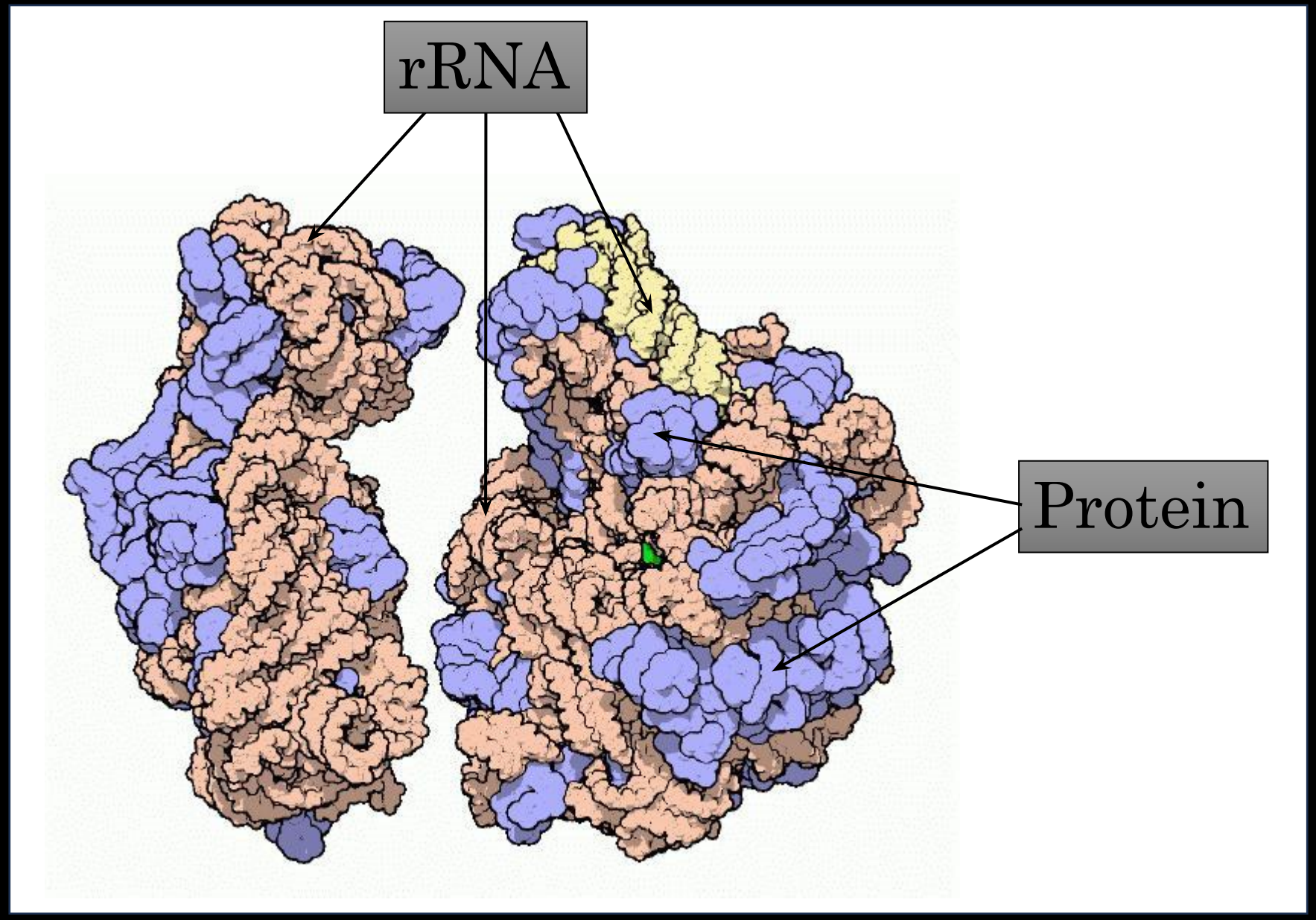
- Messenger RNA (mRNA) – ultimately translated to protein (the next step)

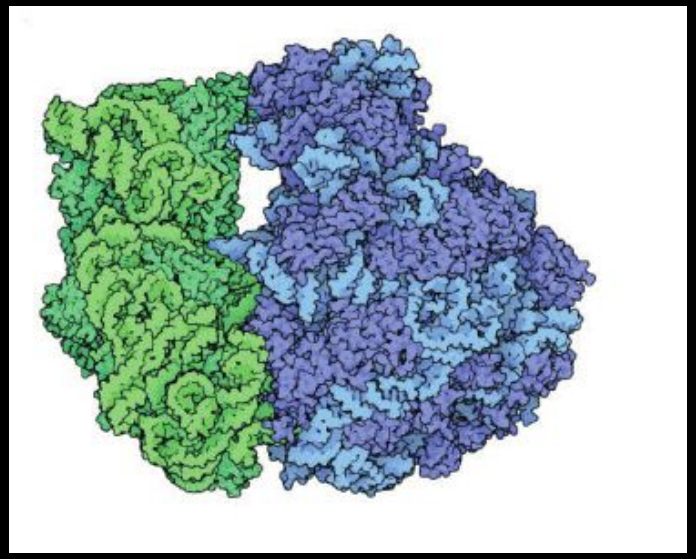
Noncoding RNA

- Ribosomal RNA (rRNA) – important to translation
- Transfer RNA (tRNA) – also important to translation
- About 20 other types at last count!

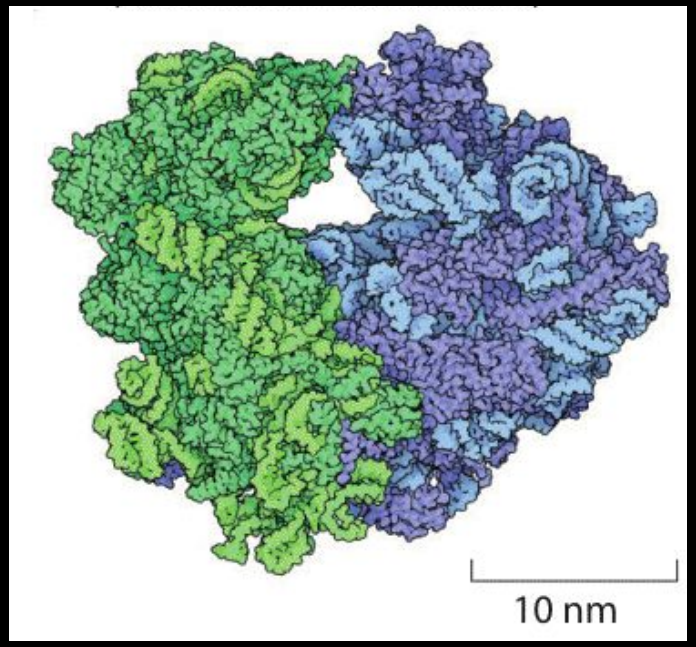
TRANSLATION: from mRNA to protein

Key player: the ribosome



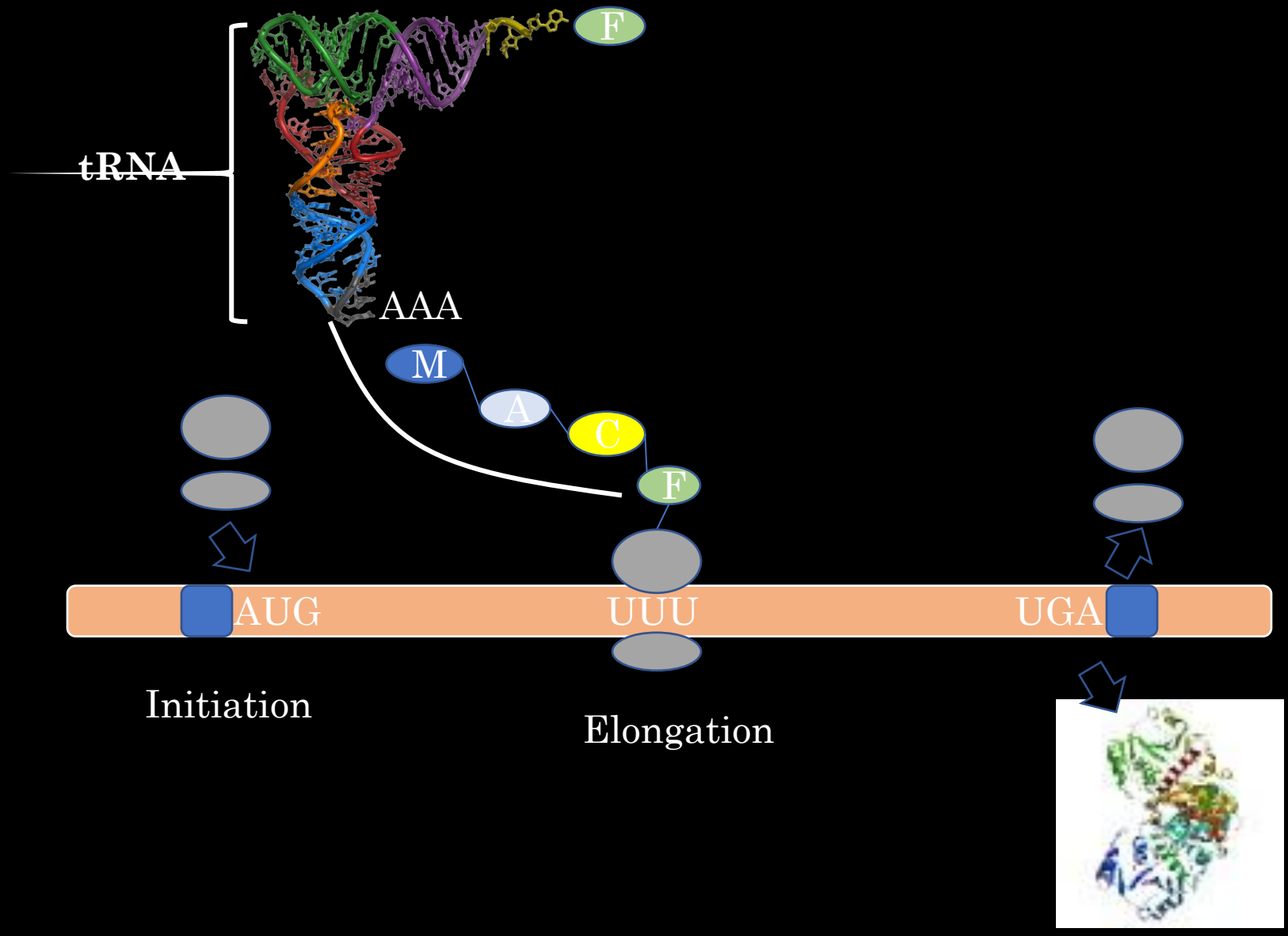


Prokaryotic ribosome
~45 proteins
3 rRNAs



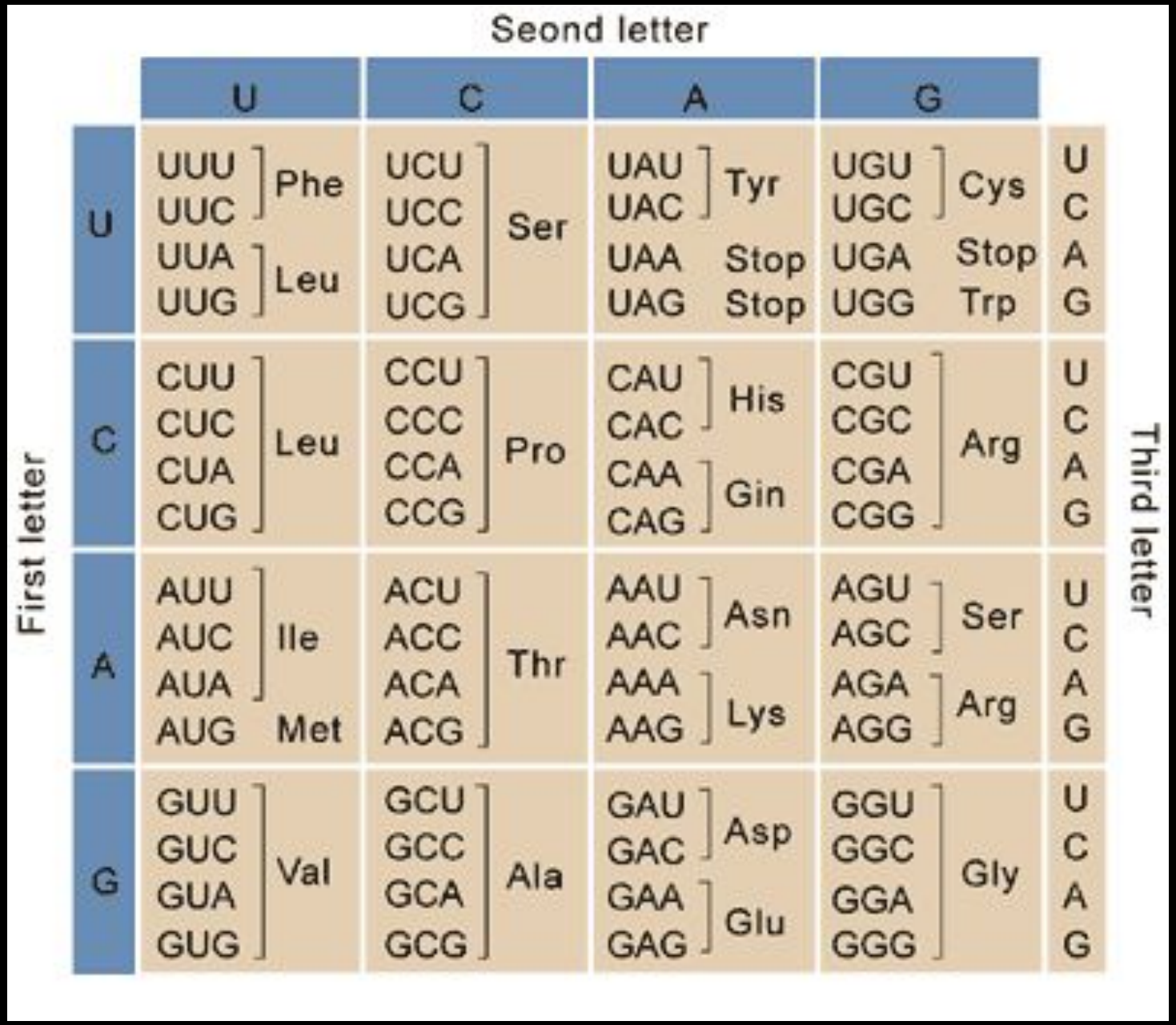
Eukaryotic ribosome
~80 proteins
4 RNAs

http://book.bionumbers.org/how-big-are-the-molecular-machines-of-the-central-dogma/



http://commons.wikimedia.org/wiki/File:TRNA-Phe_yeast_1ehz.png

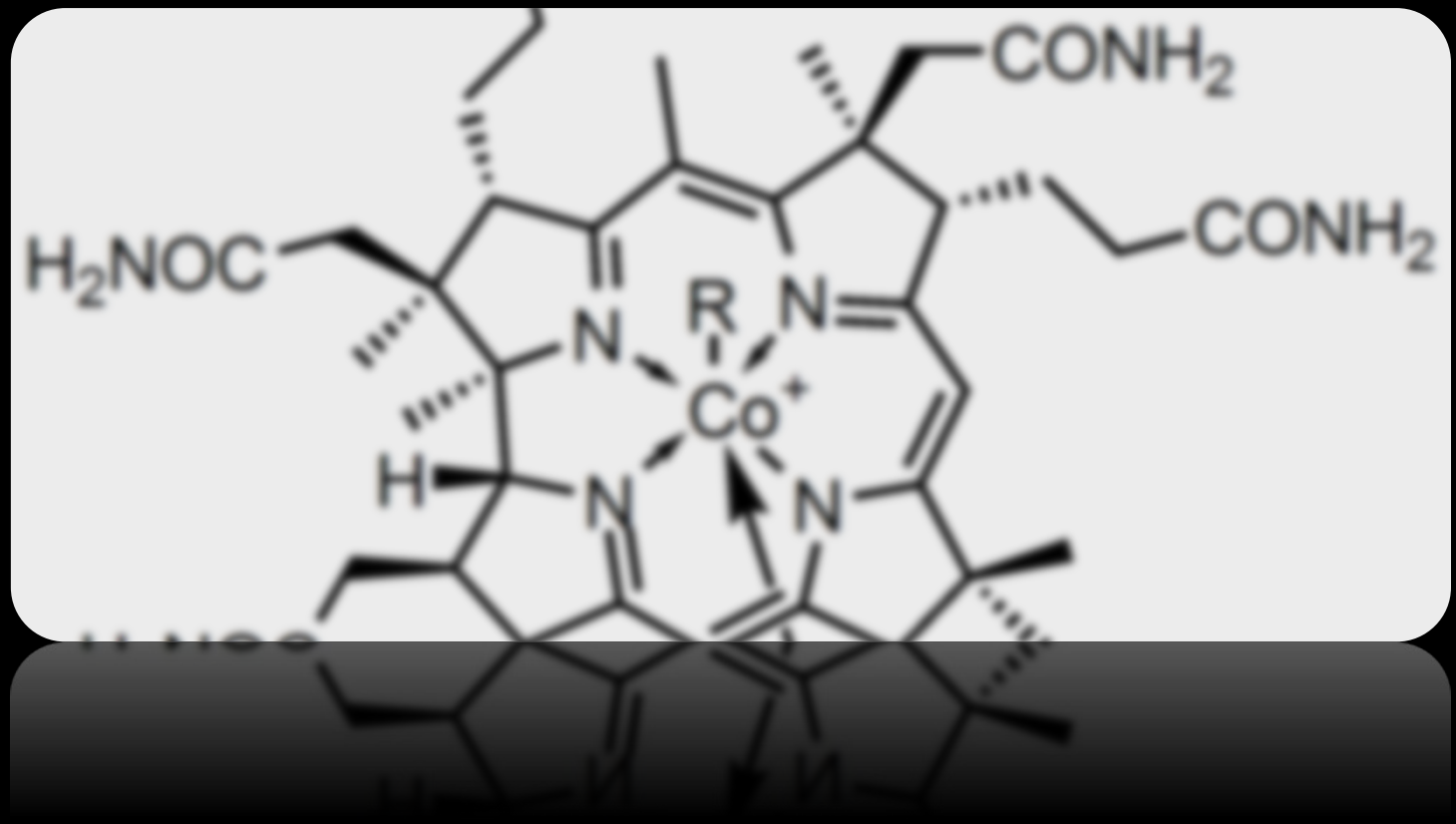
The triplet genetic code (standard version)



First letter	Seond letter: U	Seond letter: C	Seond letter: A	Seond letter: G	Third letter
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gin	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gin	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

http://upload.wikimedia.org/wikipedia/commons/2/21/06_chart_pu3.png

Goodsell (2009) *Biochem Mol Biol Educ*



Biochemical pathways

One lecture, two systems

(1) Phenylketonuria

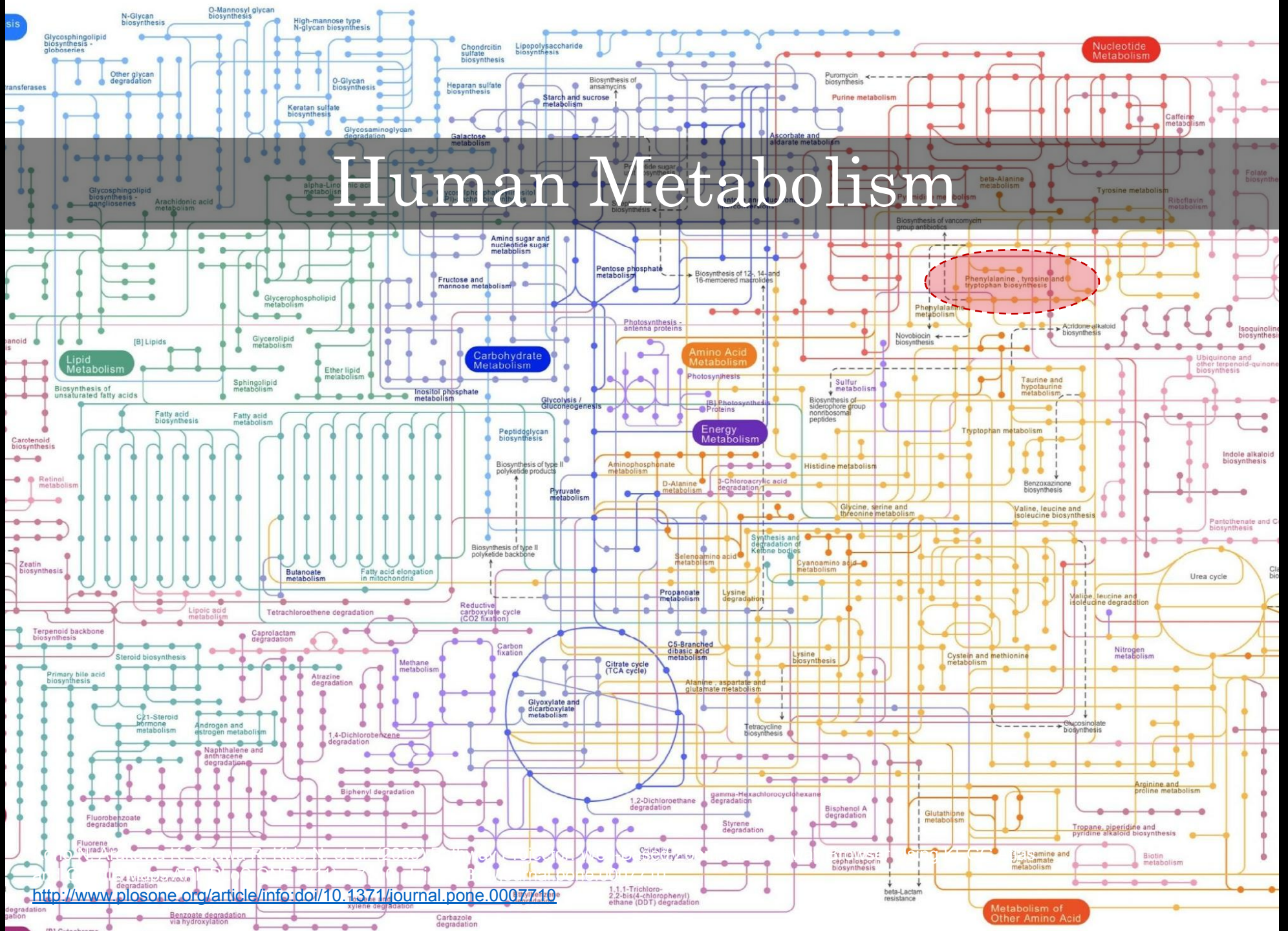
A **very important** metabolic process breaks due to mutation(s) in a critical gene, with severe health consequences

(2) Beta-lactam antibiotic resistance

Bacteria acquire a new and **very useful** function that protects them against a toxic compound

DISCLAIMER: This is a very, very simplified view of how metabolism works!!
(for example, it's not always about proteins!)

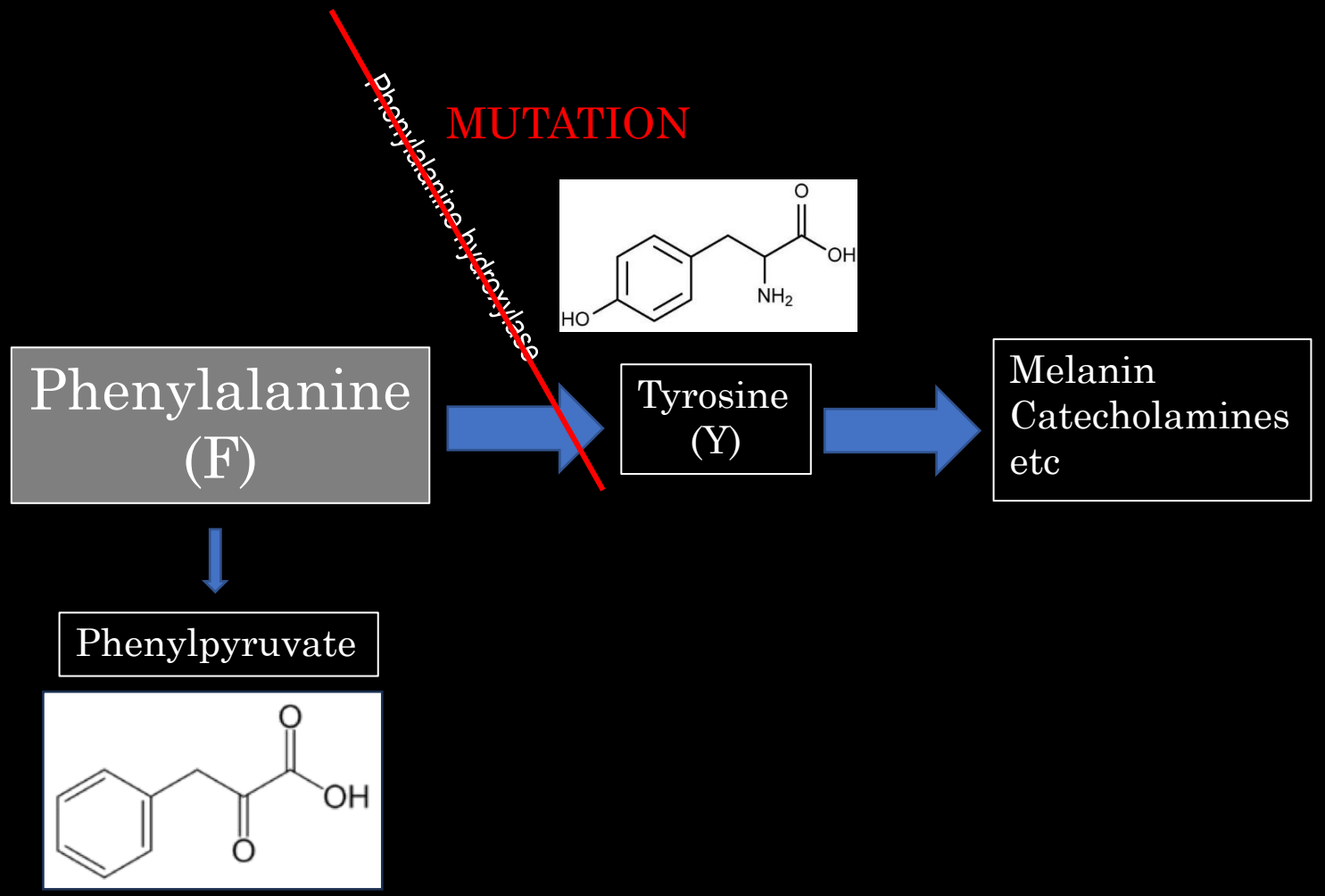
Human Metabolism



http://www.plosone.org/article/info:doi/10.1371/journal.pone.0007710

Photo: R. Beiko

Phenylketonuria (PKU)



en.wikipedia.org (user NEUROtiker)

Consequences

Too much phenylalanine and phenylpyruvate:

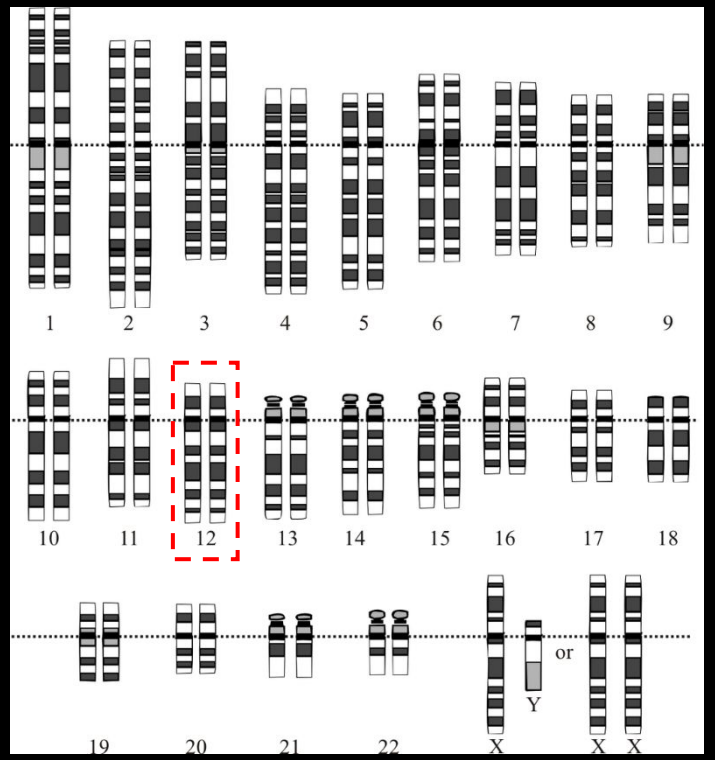
- Blocks the production of neurotransmitters (DOPA, serotonin, GABA)
- Interferes with energy production (pyruvate transport is blocked)
- Leads to impaired brain function

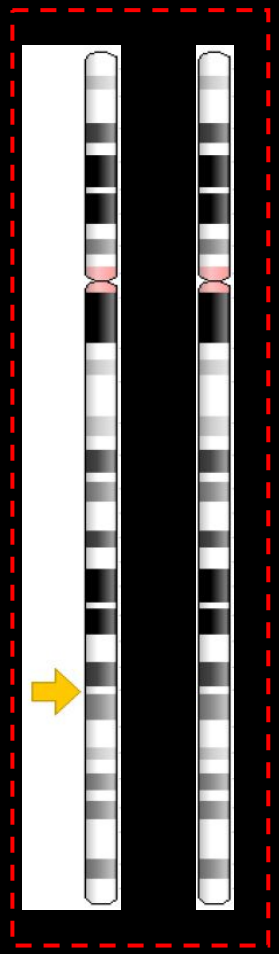
How to break a gene

Phenylalanine hydroxylase (*pah*) gene

Chen et al. (2018) *Sci Rep*

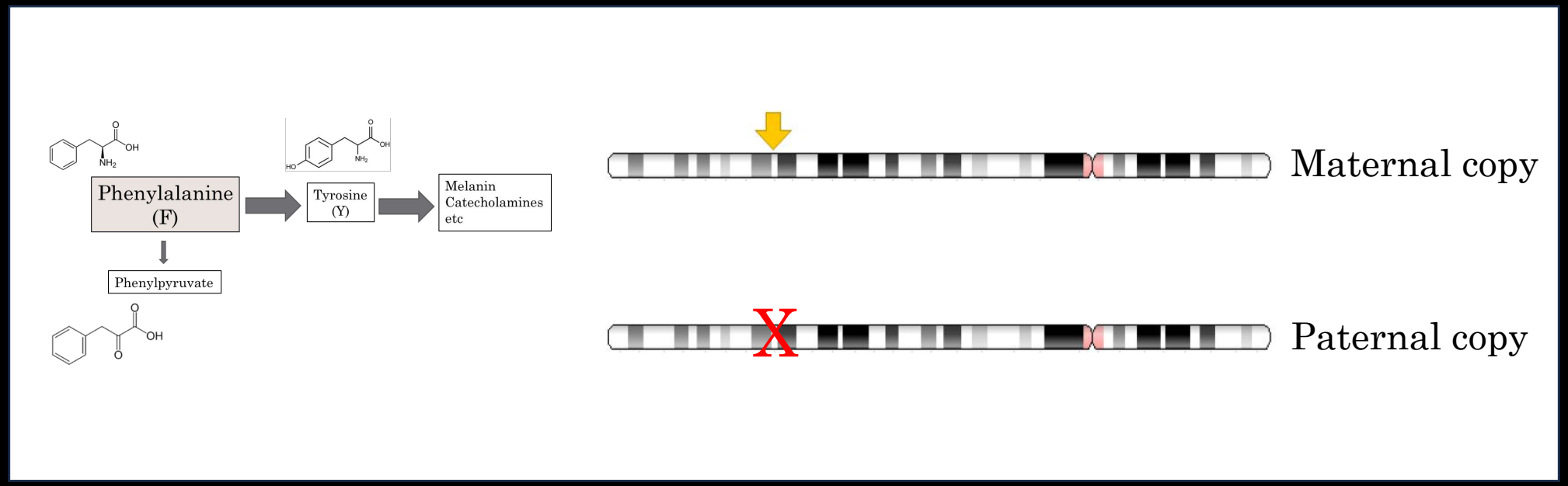
Where is the *pah* gene?





https://en.wikipedia.org/wiki/Human_genome#/media/File:Karyotype.png
Shahid (2020) *Int J Innov Sci Res Tech*

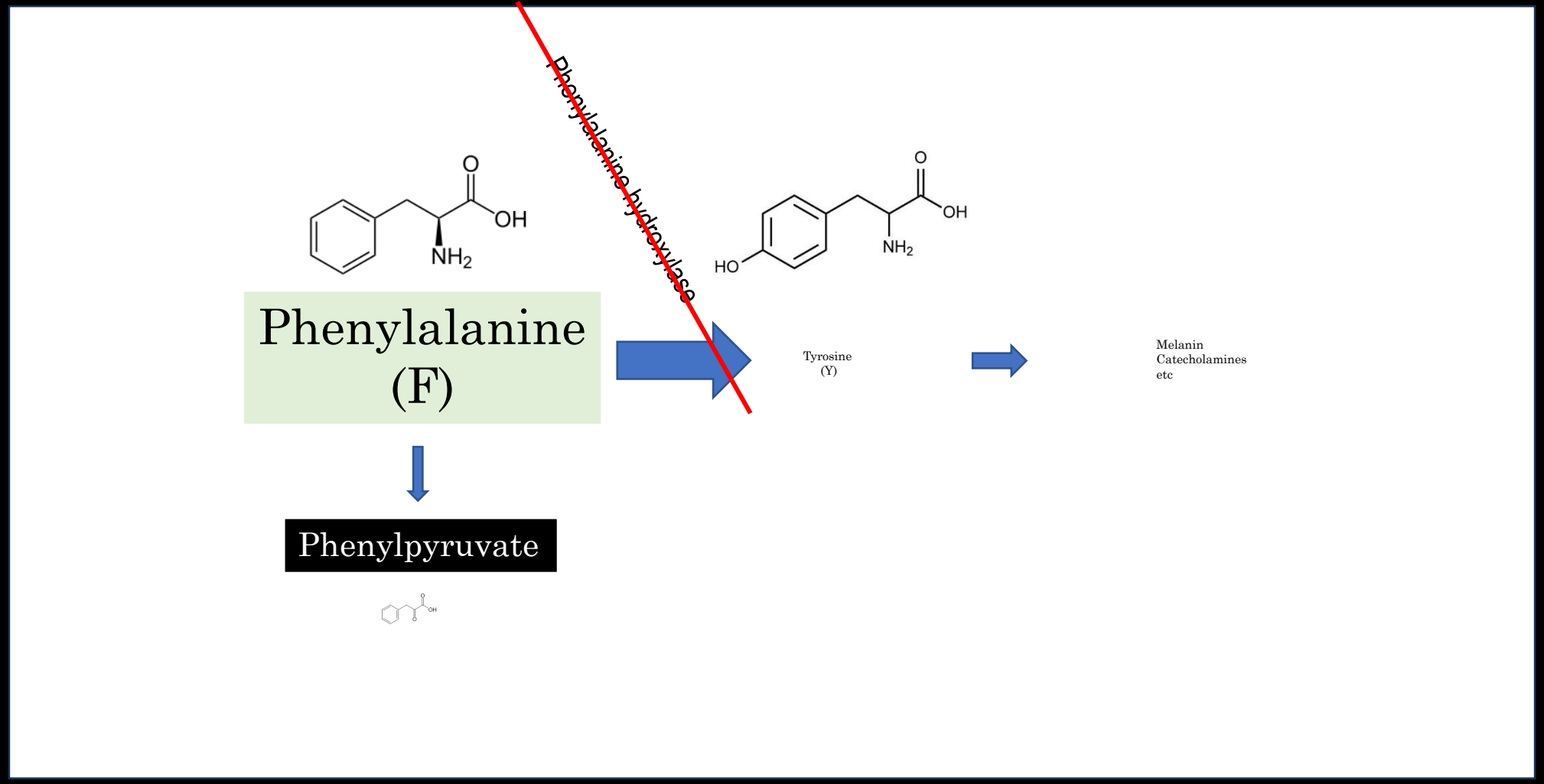
Yes mutations, no PKU



One copy is *good enough* –
if you have one "healthy" copy you don't have PKU

This is how two non-affected parents can have offspring with PKU

Treating PKU



Severely restrict phenylalanine intake = little to no phenylpyruvate concerned
(we get enough tyrosine from our diet!)

en.wikipedia.org (user NEUROtiker)

Treating PKU

No phenylalanine-containing foods

No aspartame

http://bcbiologypku.wikispaces.com/

PKU is completely treatable, **if** diagnosed at birth

No excess phenylalanine = no physiological consequences

Treatment must be initiated as soon as possible after birth!

Incidence of PKU:

Region / Country		Incidence of PKU
Asian Populations	China	1 : 17,000
	Japan	1 : 125,000
	Turkey	1 : 2,600
	Yemenite Jews (in Israel)	1 : 5,300
	Scotland	1 : 5,300
	Czechoslovakia	1 : 7,000
	Hungary	1 : 11,000
	Denmark	1 : 12,000
European Populations	France	1 : 13,500
	Norway	1 : 14,500
	United Kingdom	1 : 14,300
	Italy	1 : 17,000
	Canada	1 : 22,000
	Finland	1 : 200,000
Arabic Populations		Up to 1 : 6,000
Oceania	Australia	1: 10,000

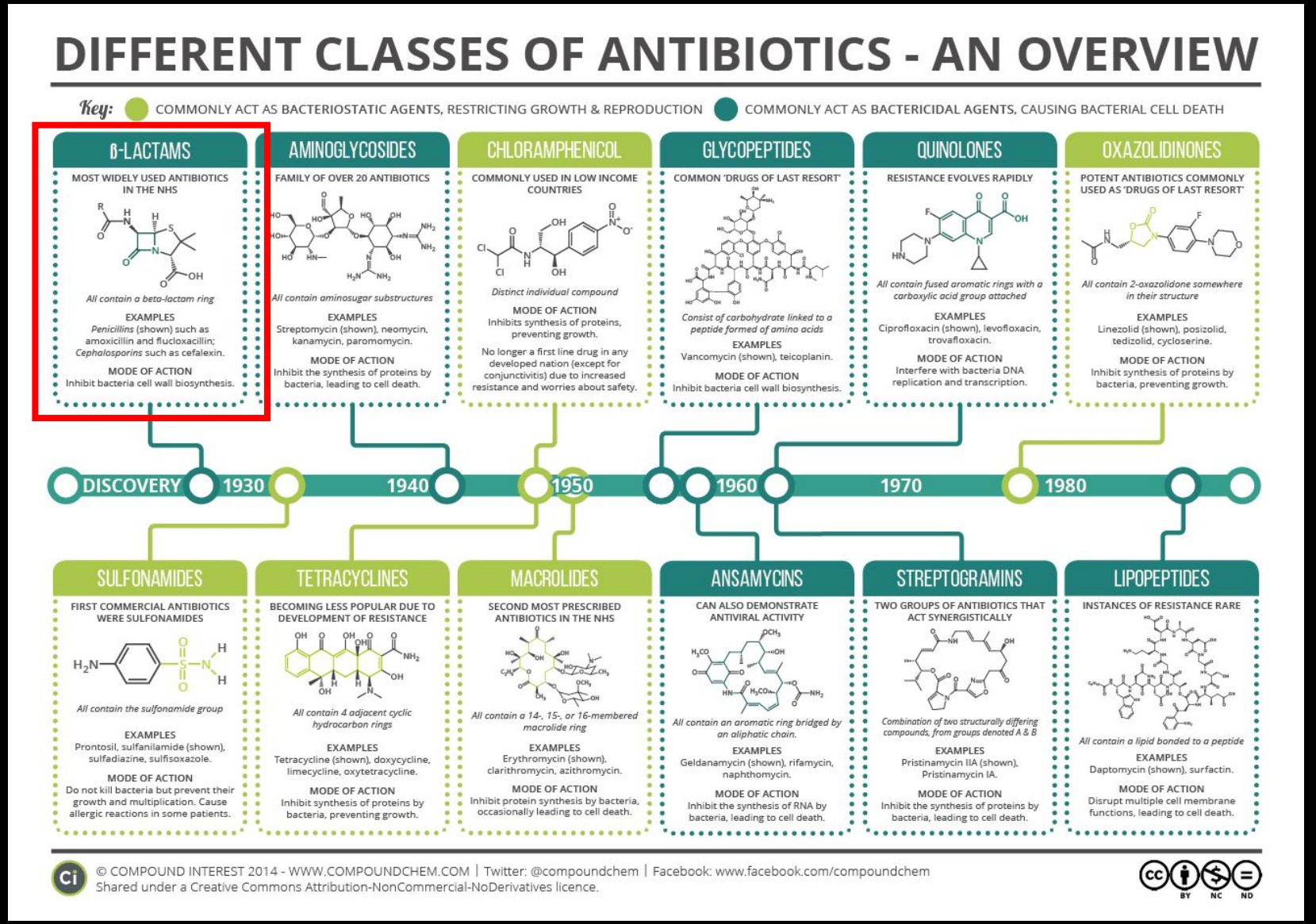
Williams et al. (2008) *Clin Biochem Rev*

Guthrie test
for phenylalanine in
blood

Photo: R. Beiko

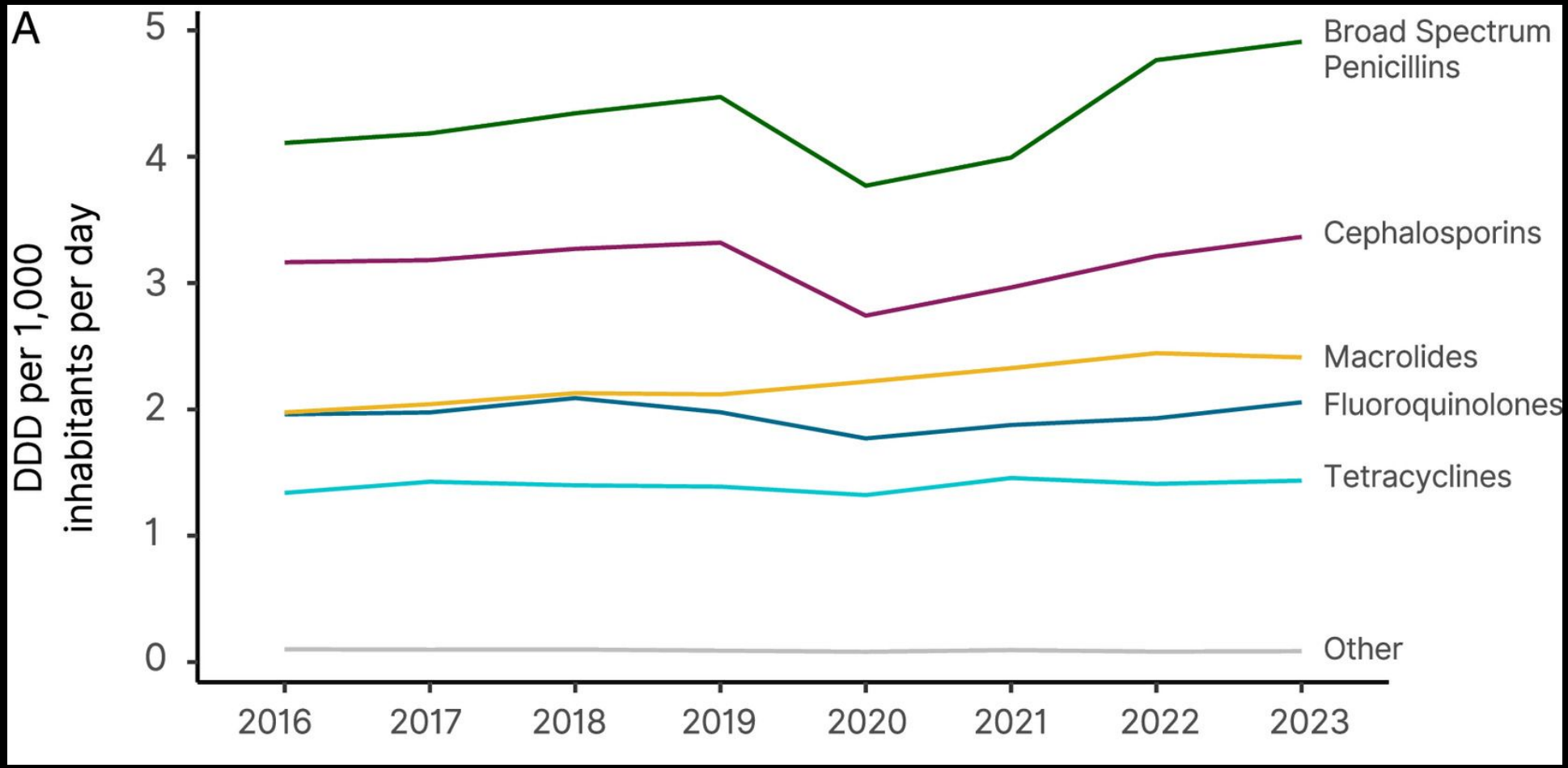
http://commons.wikimedia.org/wiki/File:Penicillin_cures_gonorrhea.jpg

Antimicrobial resistance



https://www.compoundchem.com/2014/09/08/antibiotics/

Beta-lactams are majority of a antibiotics used



Klein, Eili Y., et al. "Global trends in antibiotic consumption during 2016–2023 and future projections through 2030." Proceedings of the National Academy of Sciences 121.49 (2024): e2411919121.

Antimicrobial Resistance impacts all antimicrobials

- Clinical Approval
- Resistance First Reported
- Resistance Same Year as Approval

Spagnolo, Fabrizio, Monica Trujillo, and John J. Dennehy. "Why do antibiotics exist?." *MBio* 12.6 (2021): e01966-21.

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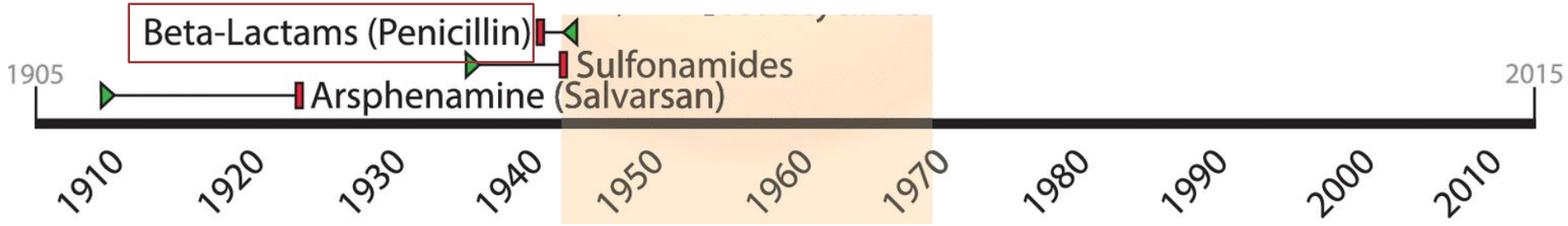

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Antimicrc

- Clinic
- Resis
- Resis

ON THE ANTIBACTERIAL ACTION OF CULTURES OF A PENICILLIUM, WITH SPECIAL REFERENCE TO THEIR USE IN THE ISOLATION OF *B. INFLUENZÆ*.

ALEXANDER FLEMING, F.R.C.S.

From the Laboratories of the Inoculation Department, St Mary's Hospital, London.

Received for publication May 10th, 1929.

WHILE working with staphylococcus variants a number of culture-plates were set aside on the laboratory bench and examined from time to time. In the examinations these plates were necessarily exposed to the air and they became contaminated with various micro-organisms. It was noticed that around a large colony of a contaminating mould the staphylococcus colonies became transparent and were obviously undergoing lysis (see Fig. 1).

Subcultures of this mould were made and experiments conducted with a view to ascertaining something of the properties of the bacteriolytic substance which had evidently been formed in the mould culture and which had diffused into the surrounding medium. It was found that broth in which the mould had been grown at room temperature for one or two weeks had acquired marked inhibitory, bactericidal and bacteriolytic properties to many of the more common pathogenic bacteria.

LETTERS TO THE EDITORS

The Editors do not hold themselves responsible for opinions expressed by their correspondents. They cannot undertake to return, or to correspond with the writers of, rejected manuscripts intended for this or any other part of NATURE. *No notice is taken of anonymous communications.*

IN THE PRESENT CIRCUMSTANCES, PROOFS OF "LETTERS" WILL NOT BE SUBMITTED TO CORRESPONDENTS OUTSIDE GREAT BRITAIN.

An Enzyme from Bacteria able to Destroy Penicillin

FLEMING[1] noted that the growth of *B. coli* and a number of other bacteria belonging to the coli-typhoid group was not inhibited by penicillin. This observation has been confirmed. Further work has been done to find the cause of the resistance of these organisms to the action of penicillin.

An extract of *B. coli* was made by crushing a suspension of the organisms in the bacterial crushing mill of Booth and Green[2]. This extract was found to contain a substance destroying the growth-inhibiting property of penicillin. The destruction took place on *B. coli*, it was not necessary to crush the organism in the bacterial mill in order to obtain the enzyme from it; the latter appeared in the culture fluid. The enzyme was also found in *M. lysodeikticus*, an organism sensitive to the action of penicillin, though less so than *Staphylococcus aureus*. Thus, the presence or absence of the enzyme in a bacterium may not be the sole factor determining its insensitivity or sensitivity to penicillin.

The tissue extracts and tissue autolysates that have been tested were found to be without action on the growth-inhibiting power of penicillin. Prof. A. D. Gardner has found staphylococcal pus to be devoid of inhibiting action, but has demonstrated a slight

Antimicrobial Resistance impacts all antimicrobials

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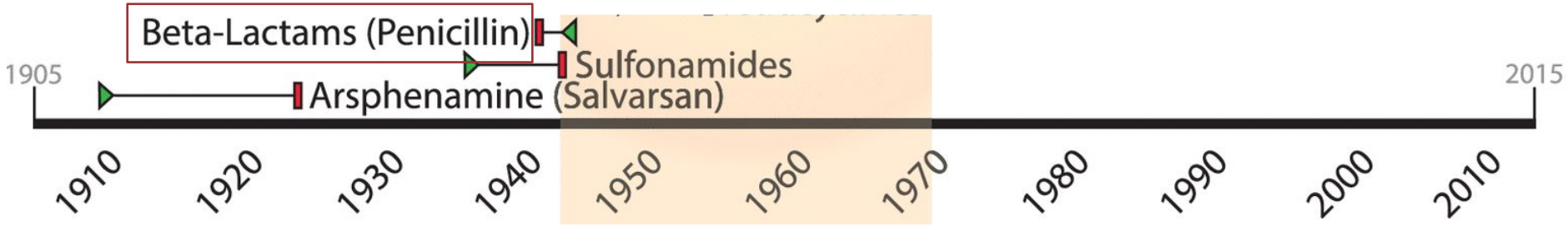
Penicillin treatment was started on 12 February 1941, with 200 mg (10 000 units) intravenously initially and then 300 mg every three hours. All the patient's urine was collected, and each morning I took it over to the Dunn Laboratory on my bicycle so that the excreted penicillin could be extracted to be used again. There I was always eagerly met by Florey and Chain and other members of the team. On the first day I was able to report that for the first time throughout his illness the patient was beginning to feel a little better. Four days later there was a striking improve-



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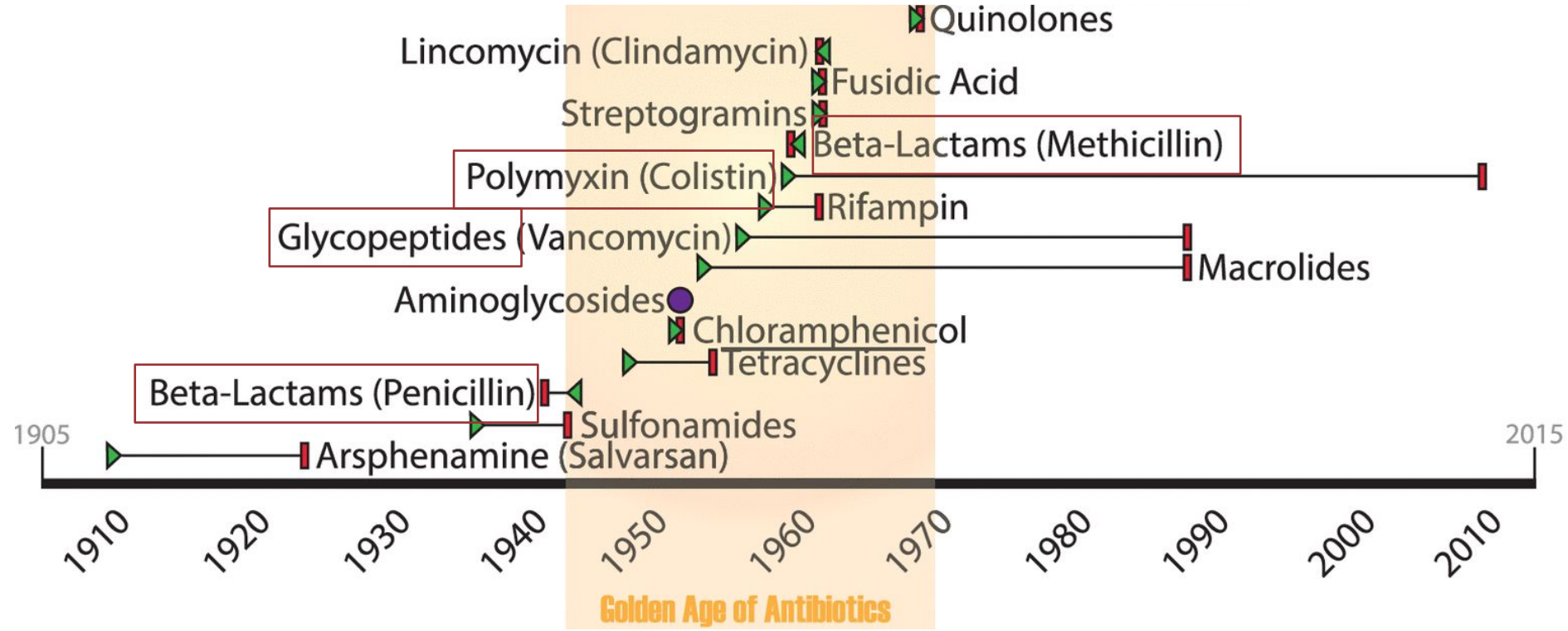

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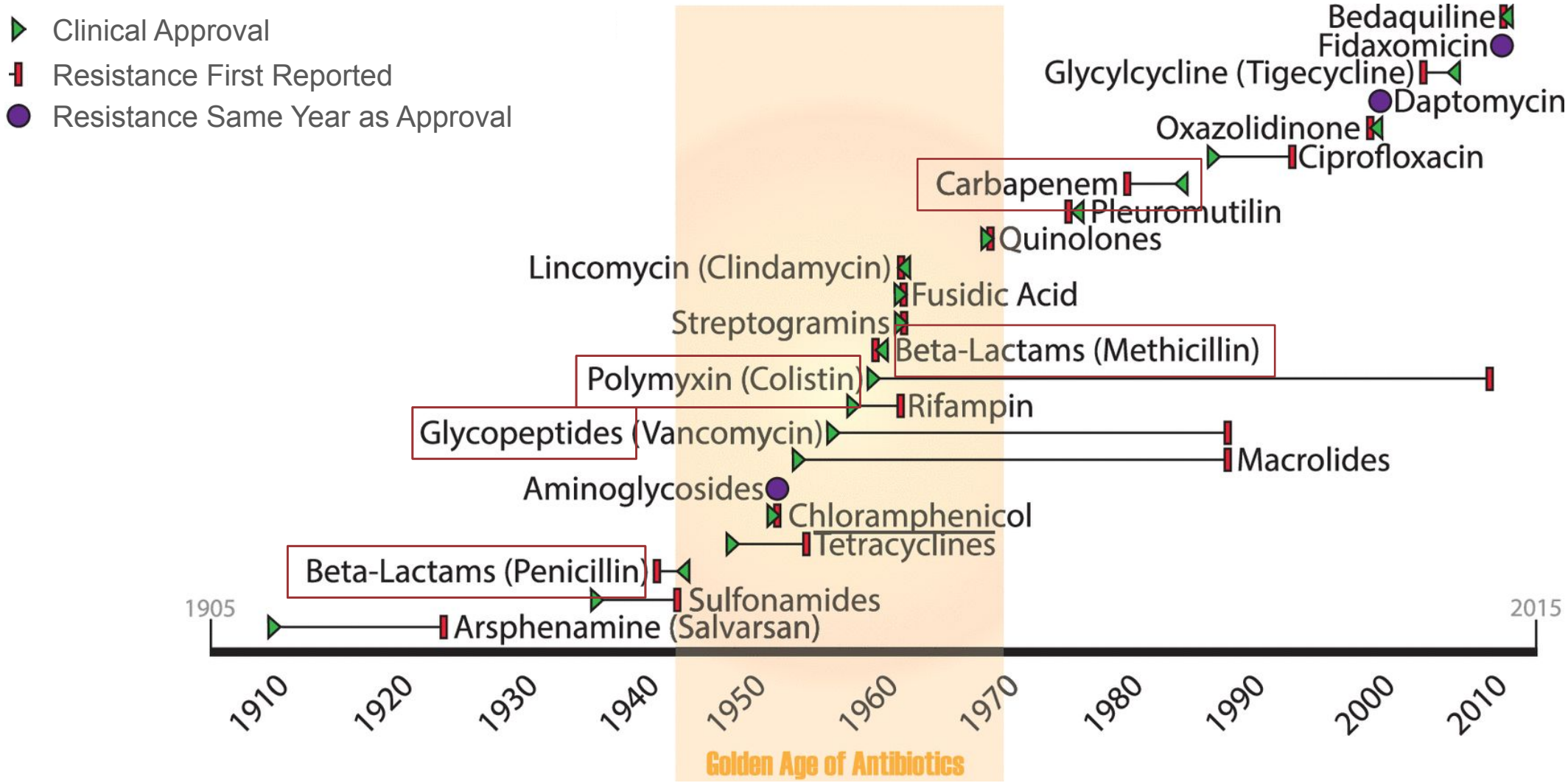

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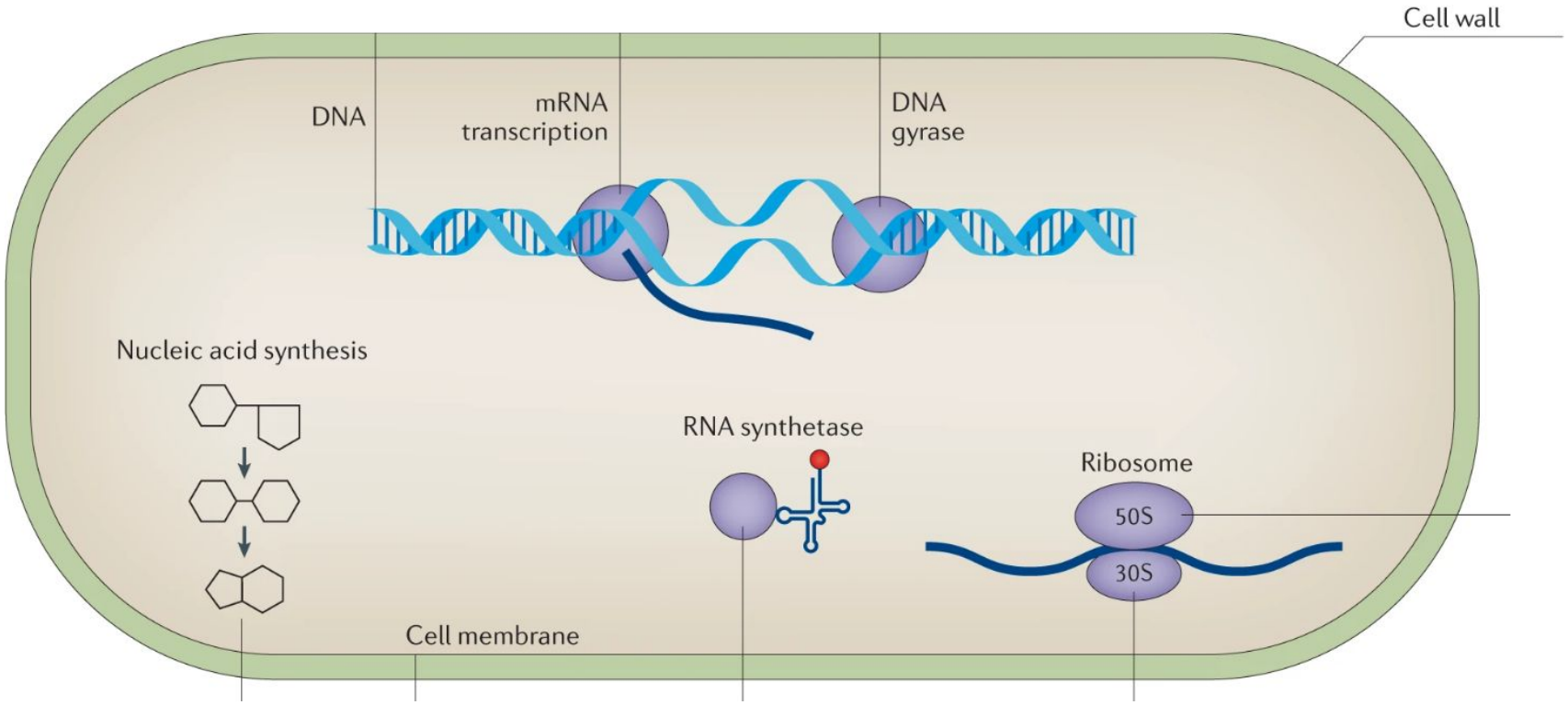
Many antibiotics - few targets



Boolchandani, Manish, Alaric W. D'Souza, and Gautam Dantas. "Sequencing-based methods and resources to study antimicrobial resistance." Nature Reviews Genetics 20.6 (2019): 356-370.

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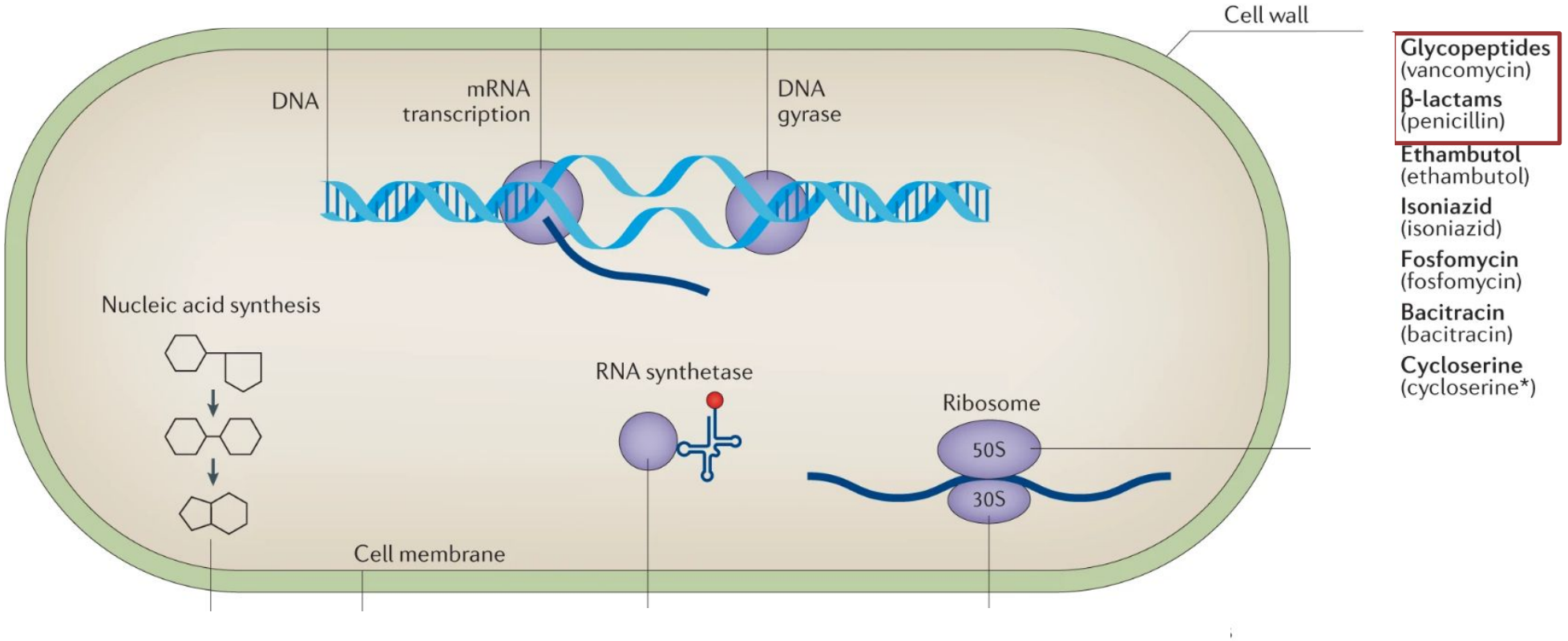

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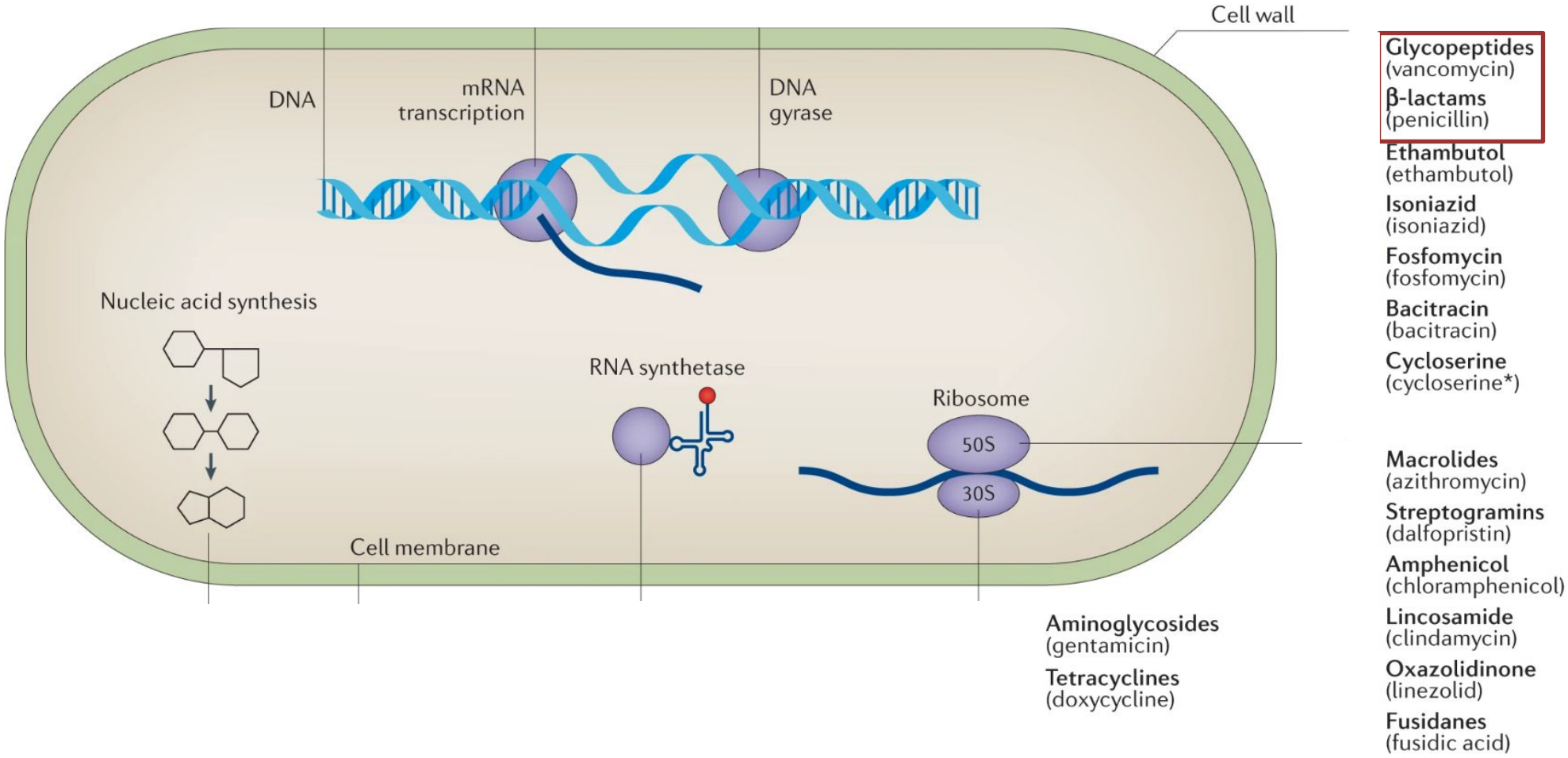

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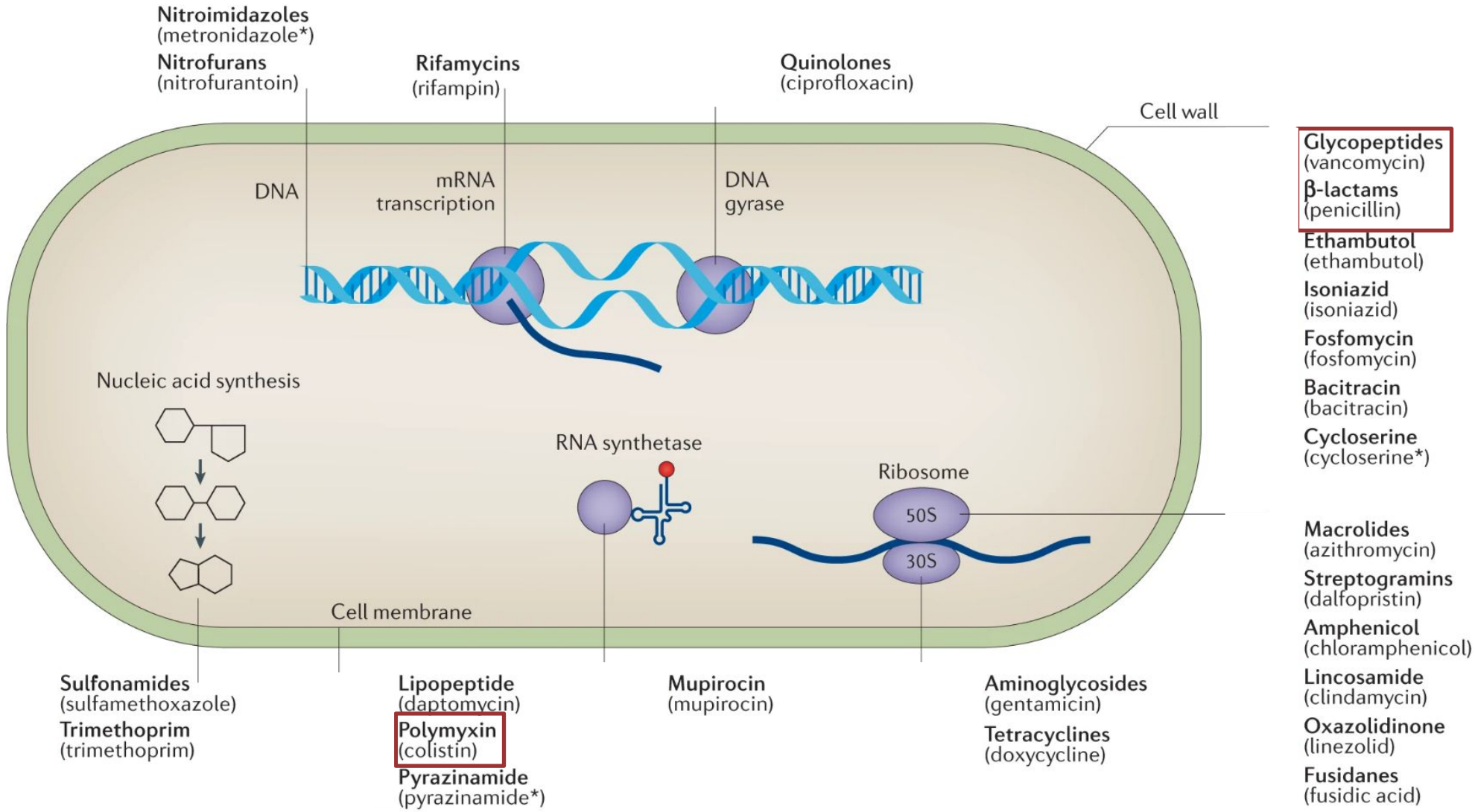

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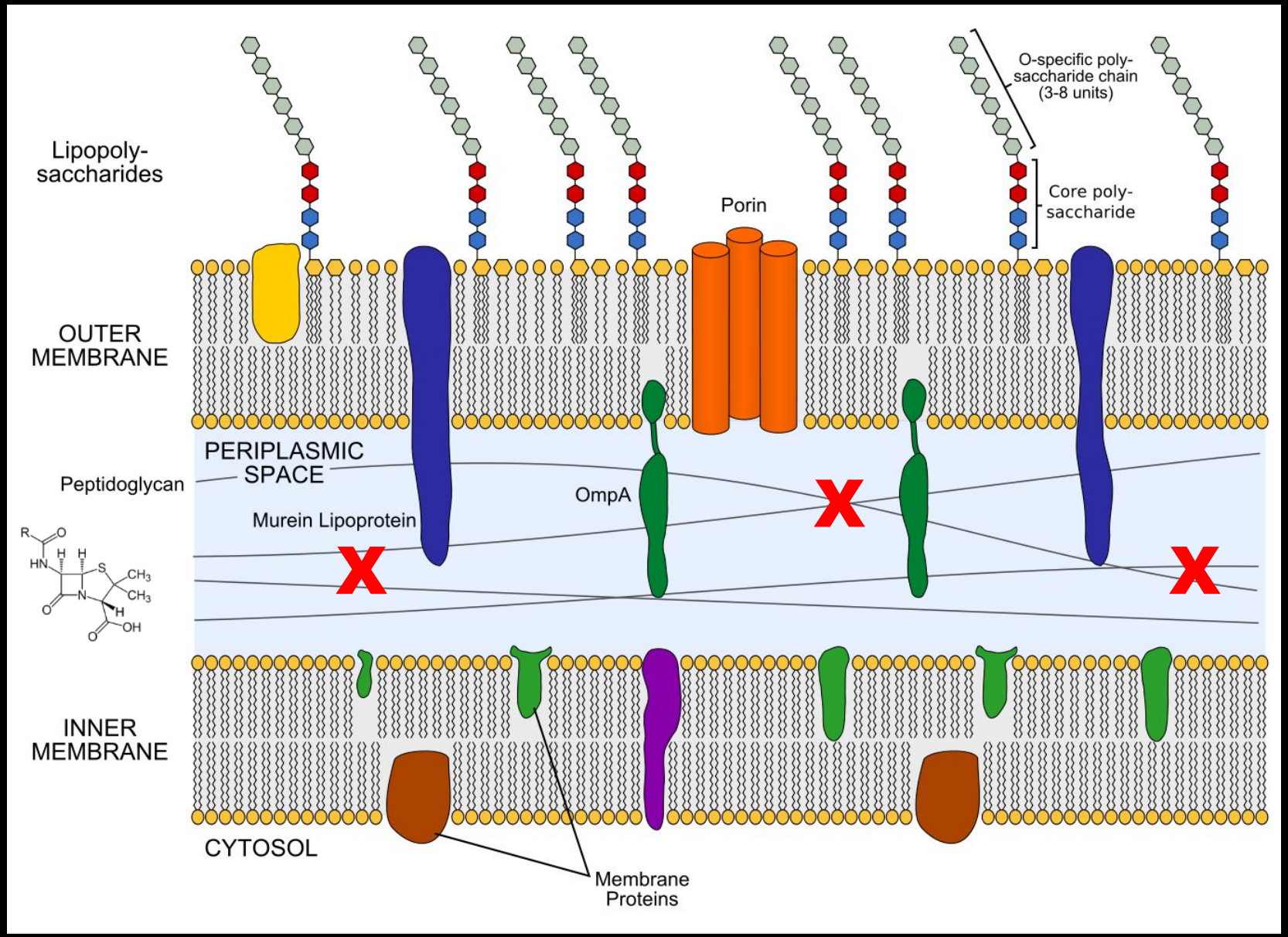
Quick disclaimer

- There are many enzymes that can break down beta-lactams
- I'll be referring to a couple of very similar but non-identical genes in a couple of different organisms

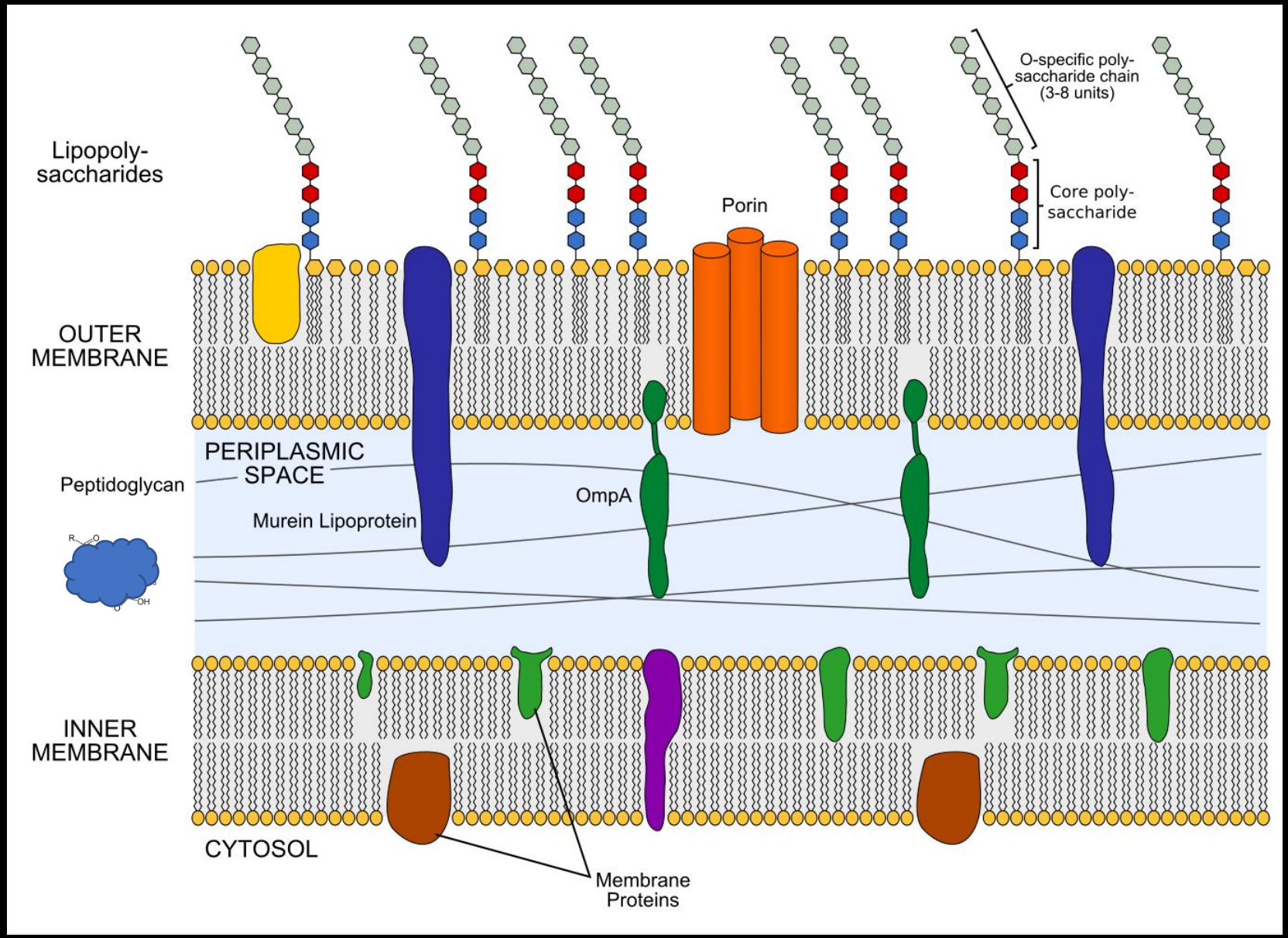
The bacterial cell wall



β-lactam antibiotics (such as penicillin) interfere with cell wall assembly

http://upload.wikimedia.org/wikipedia/commons/thumb/8/8b/Gram_negative_cell_wall.svg/2000px-Gram_negative_cell_wall.svg.png

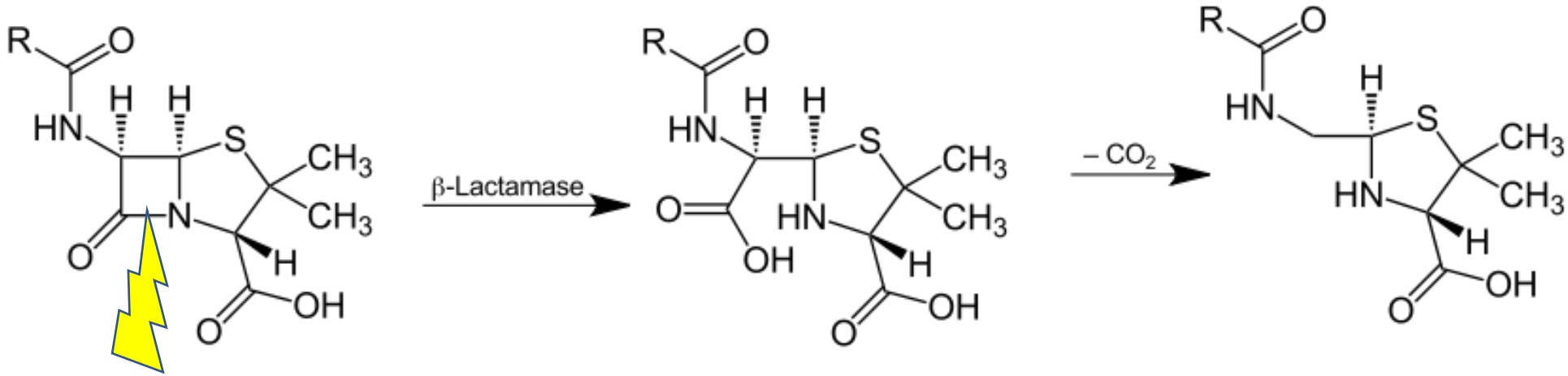
The bacterial cell wall



Cells can defend themselves with a class of proteins called **β-lactamases**

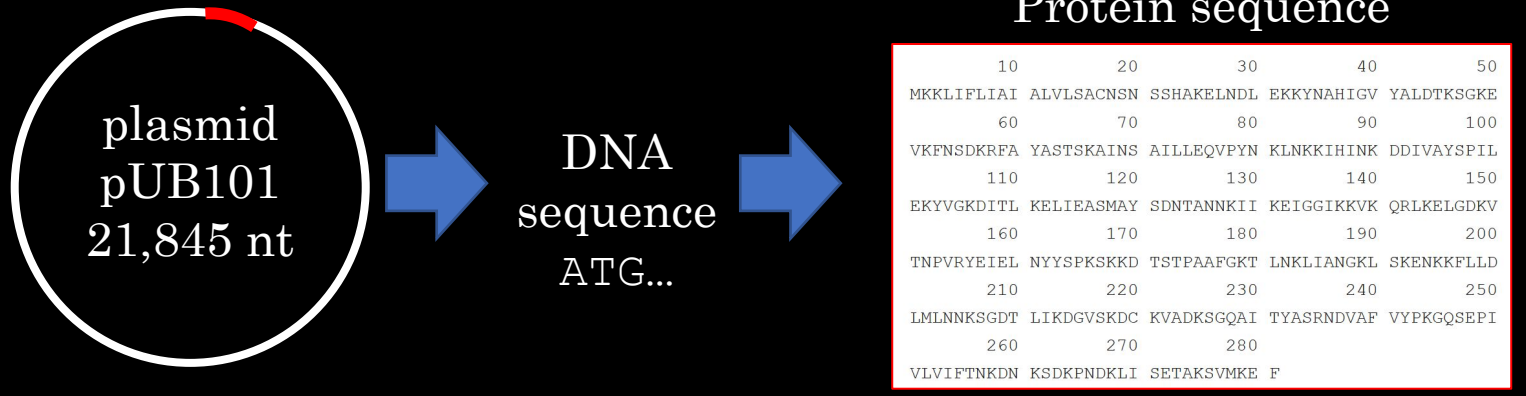
http://upload.wikimedia.org/wikipedia/commons/thumb/8/8b/Gram_negative_cell_wall.svg/2000px-Gram_negative_cell_wall.svg.png

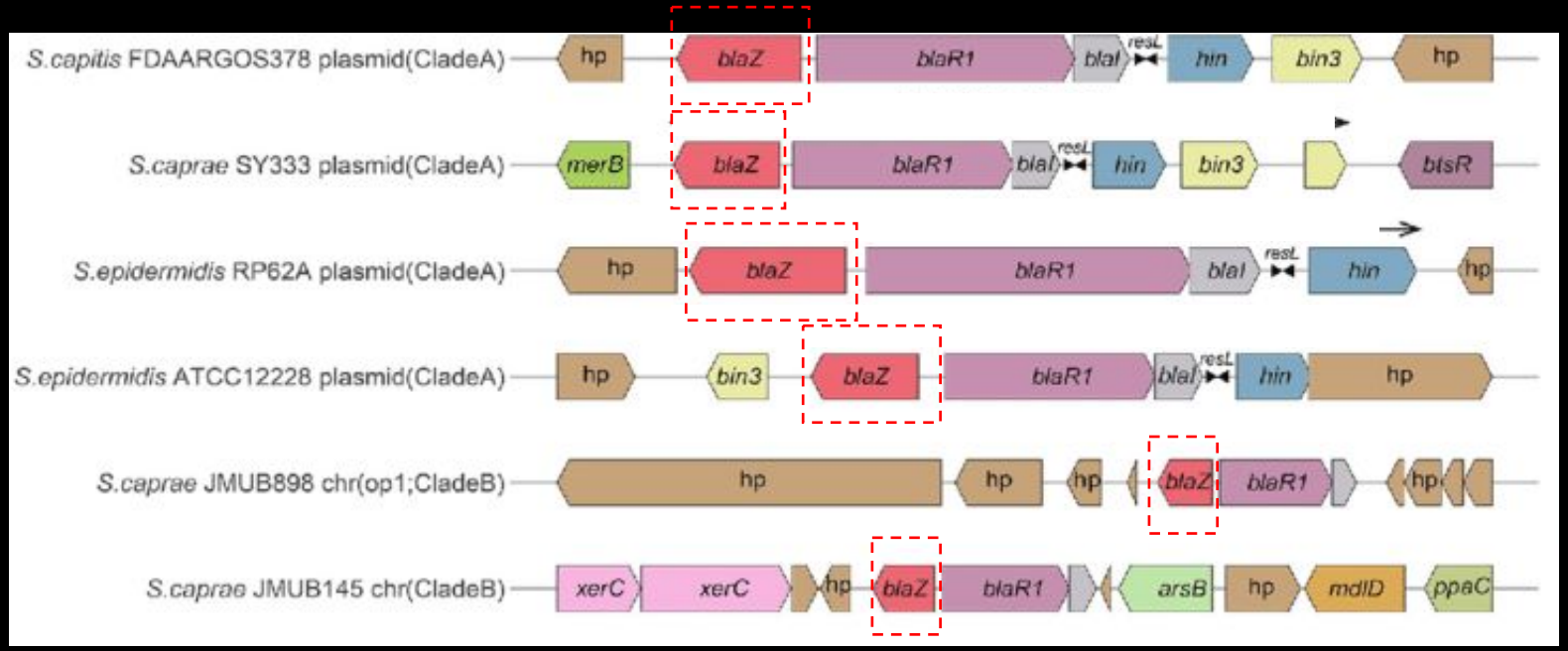
Breaking down beta-lactams



http://en.wikipedia.org/wiki/File:Lactamase_Application_V.1.svg

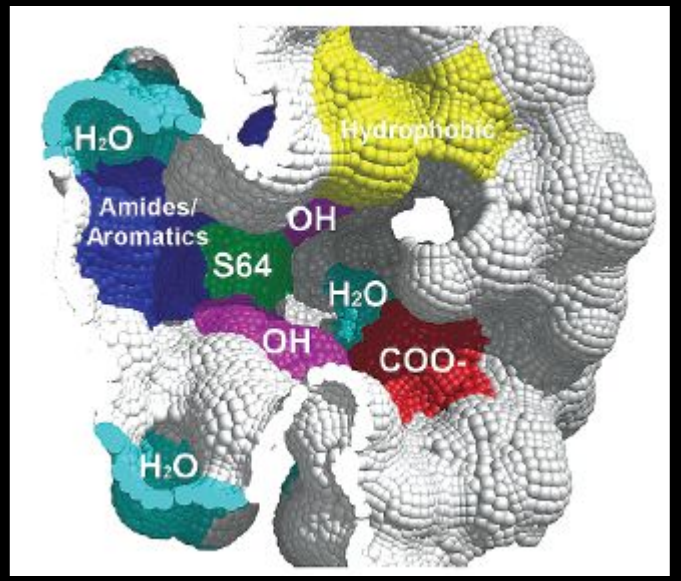
The *blaZ* gene



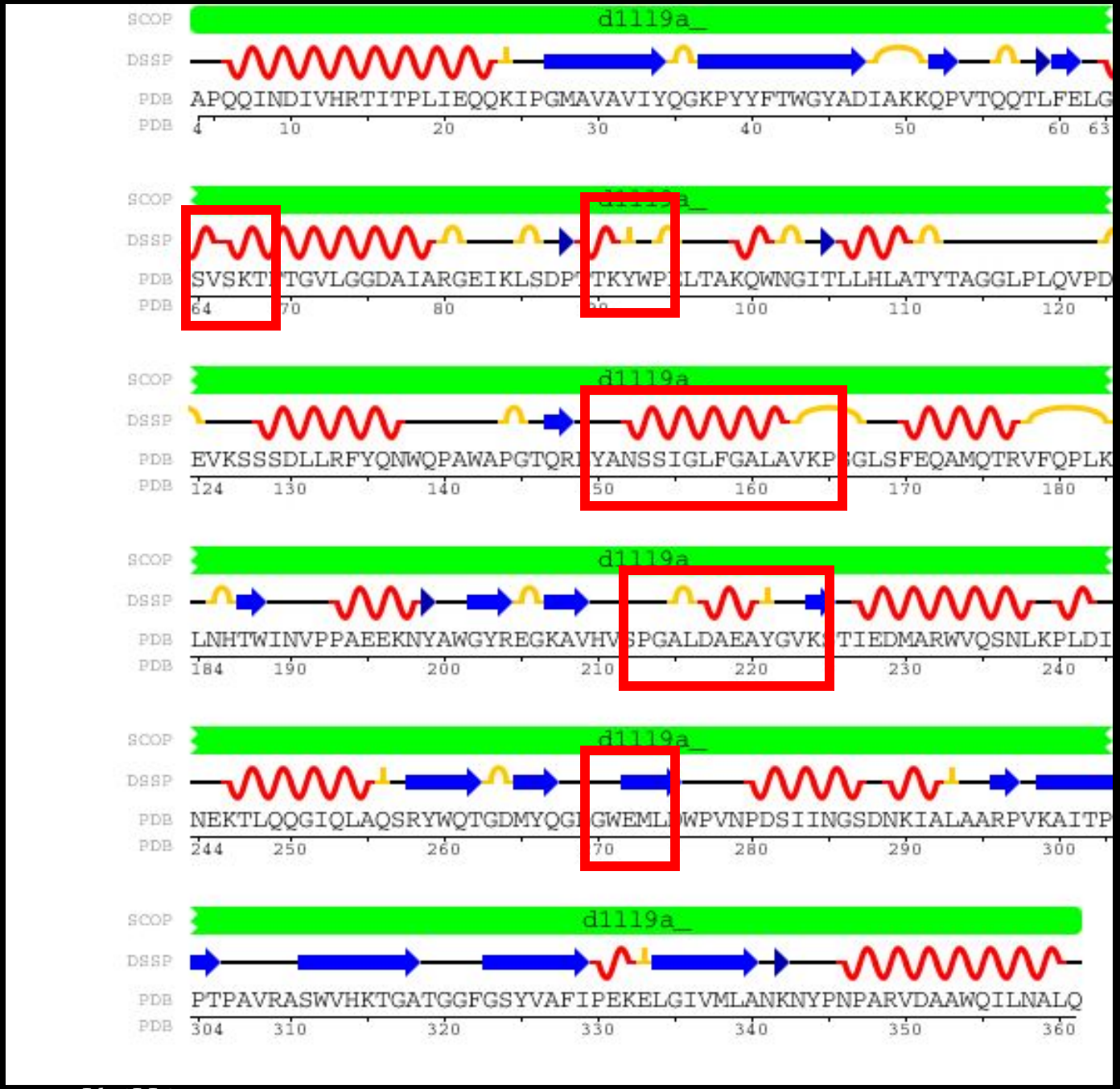


Sun et al. (2020) *Front Cell Infect Microbiol*

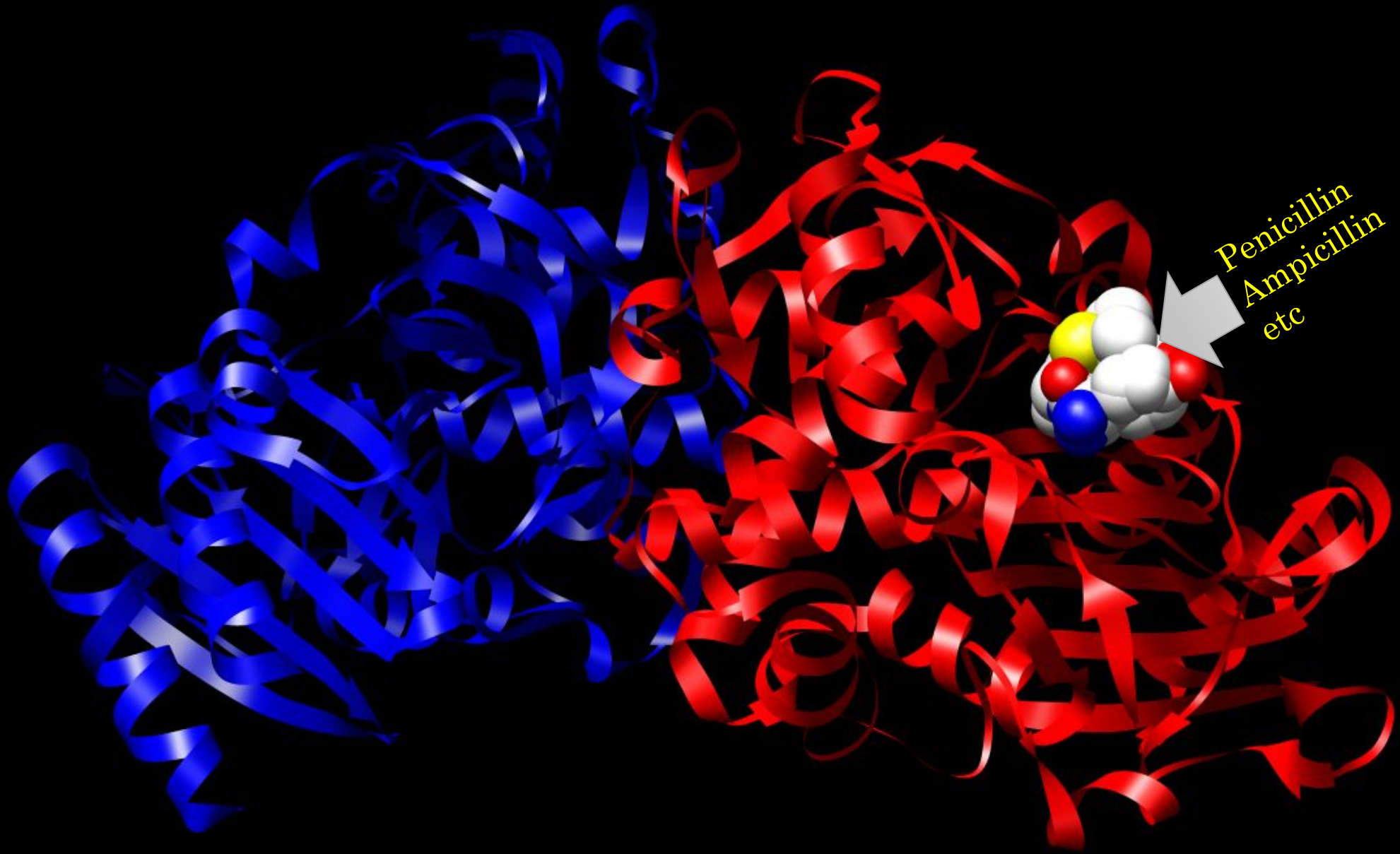
Functional sites



Powers and Sholchet, *J Med Chem* 2002

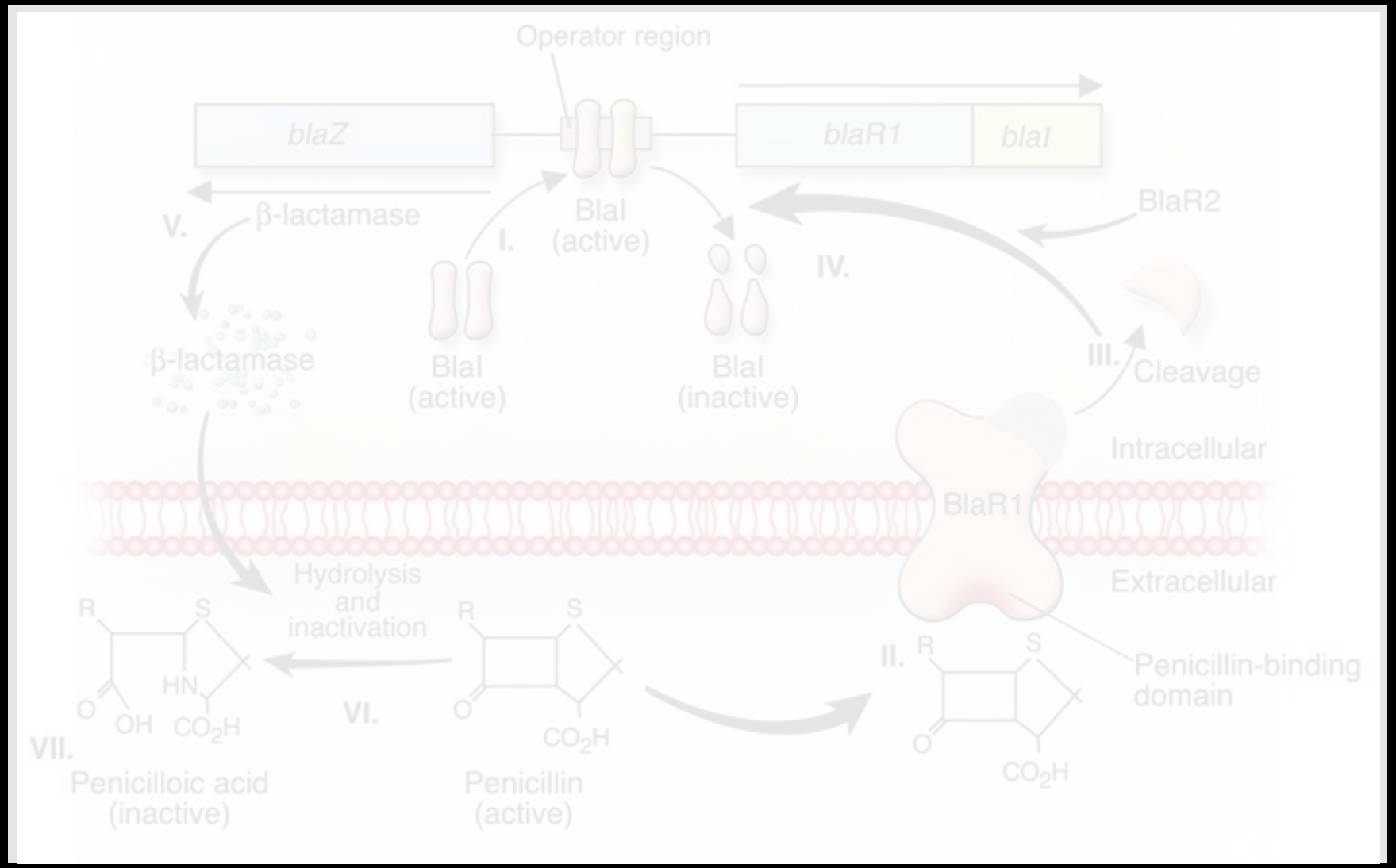


http://www.rcsb.org/pdb/explore/remediatedSequence.do?structureId=1LL9



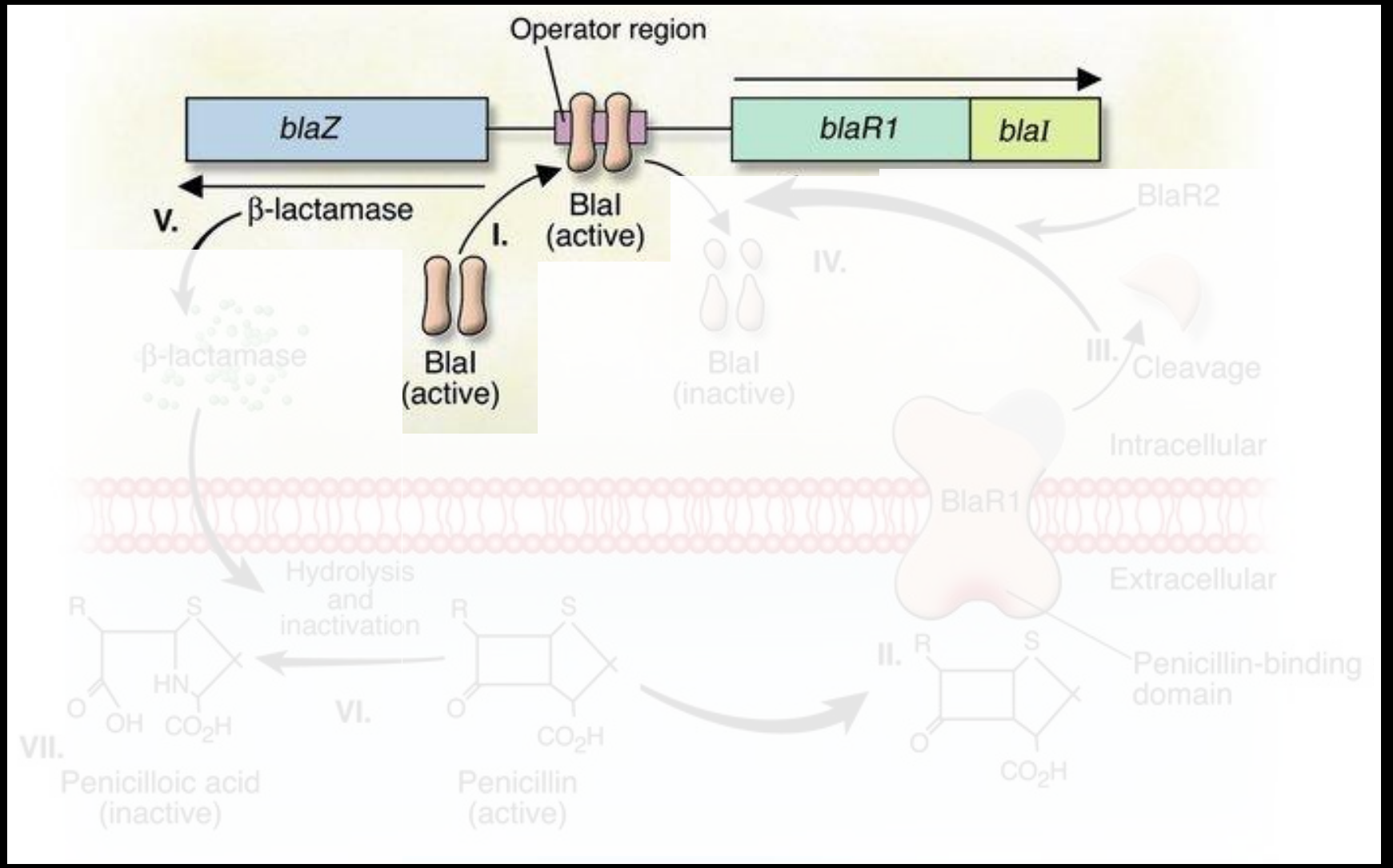
Binding antibiotics

Self-defence in several easy steps

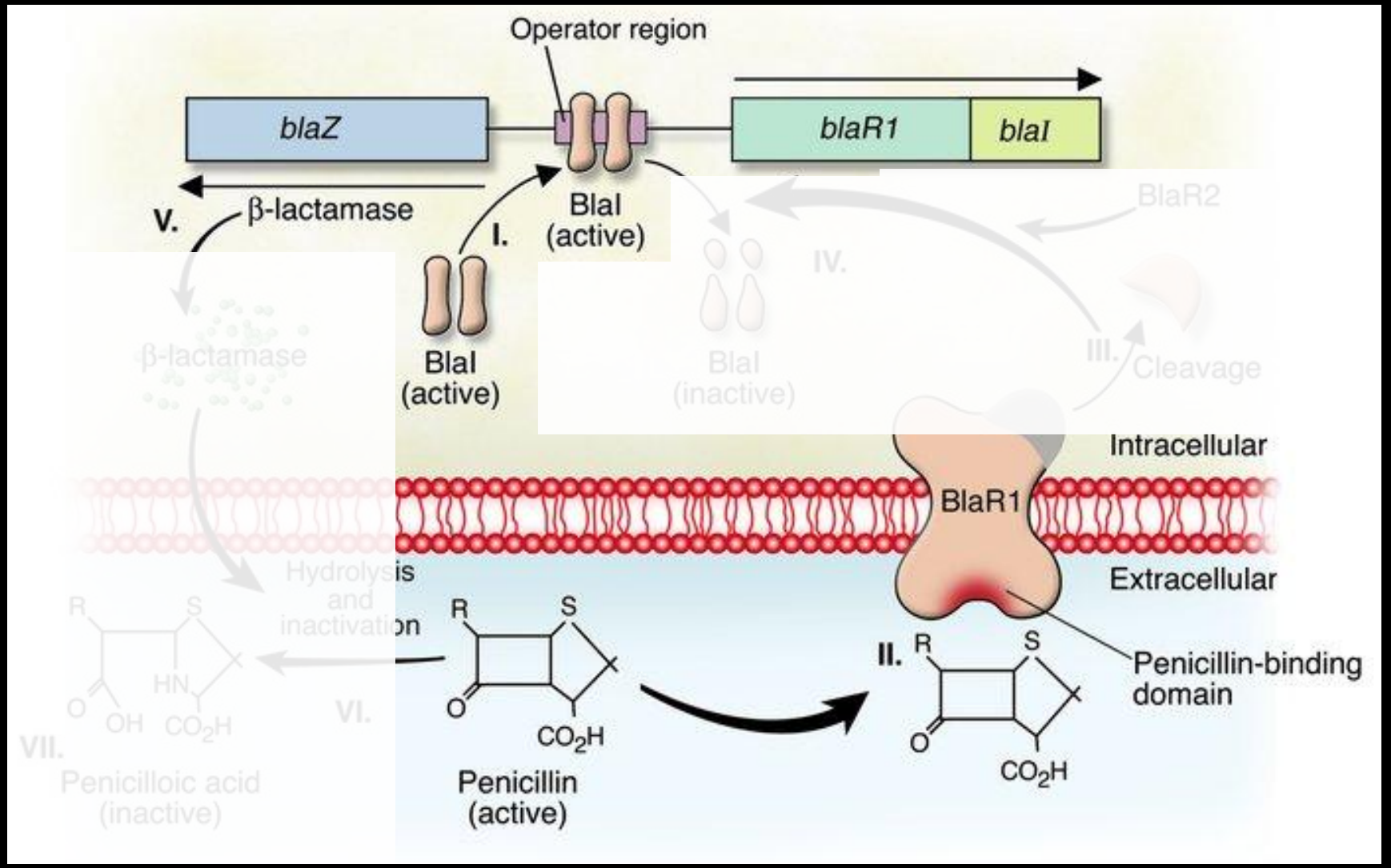


Lowy (2013) *J Clin Invest*

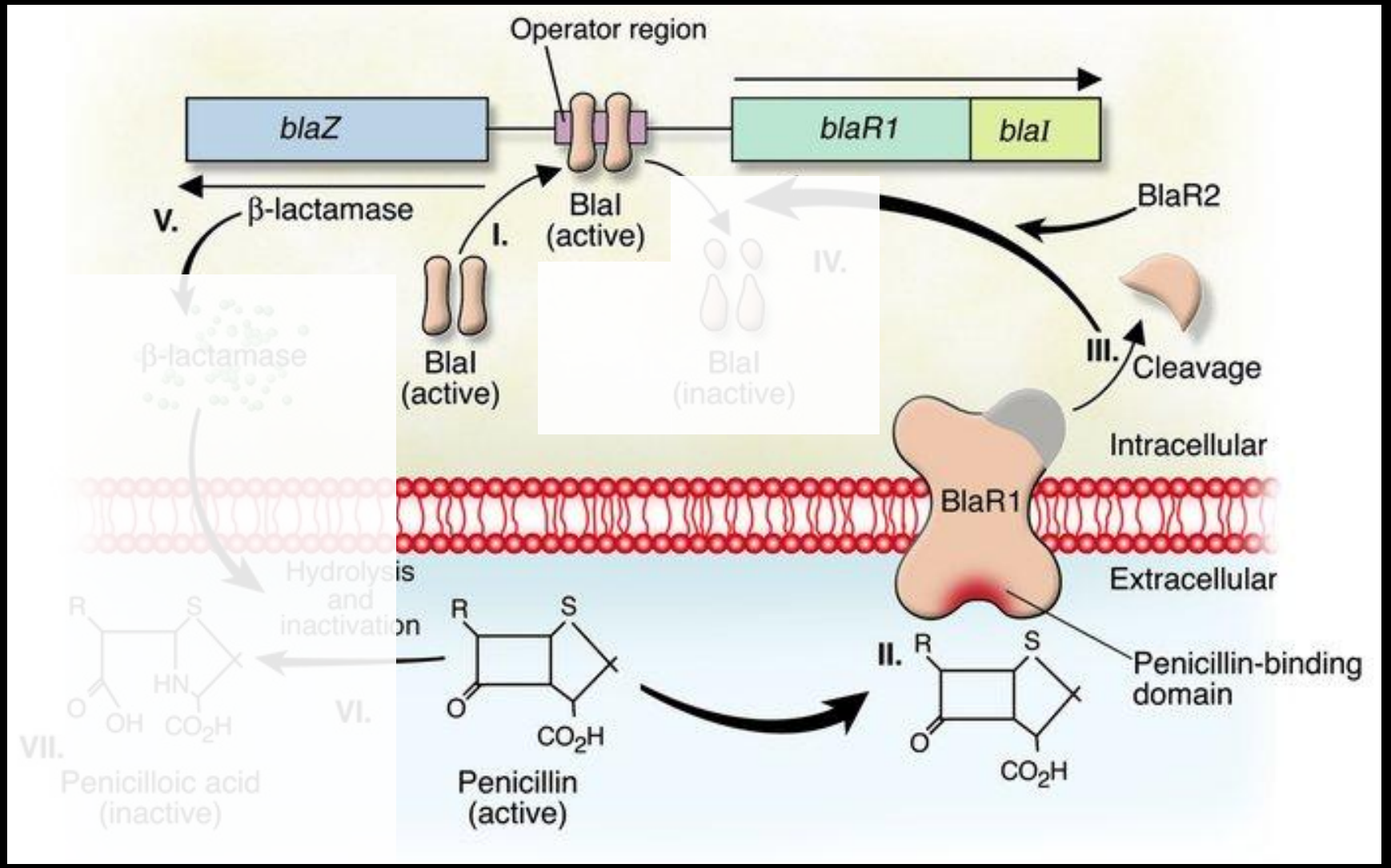
Safe and sound – BlaI keeps things quiet



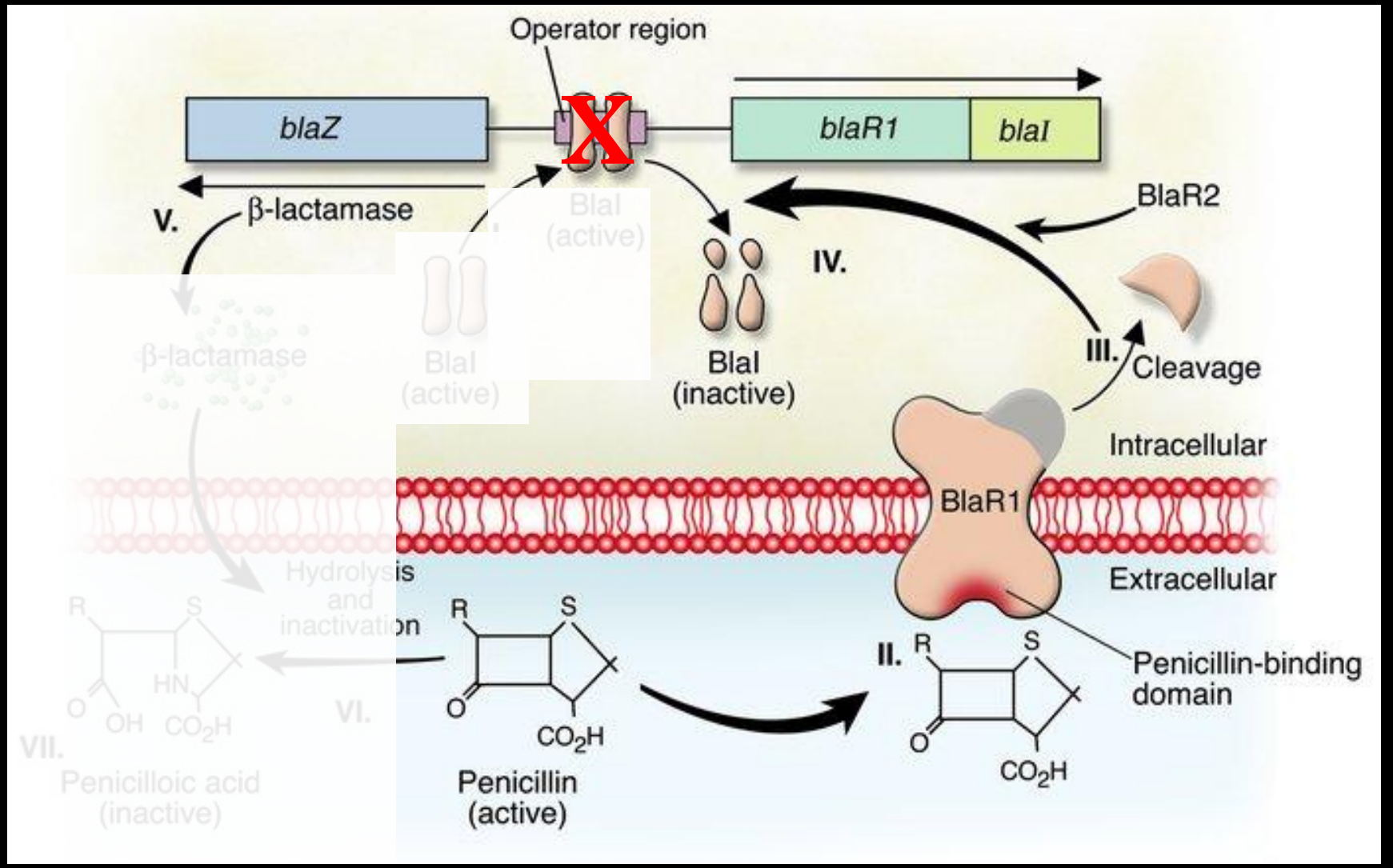
Danger! Penicillin is in the air



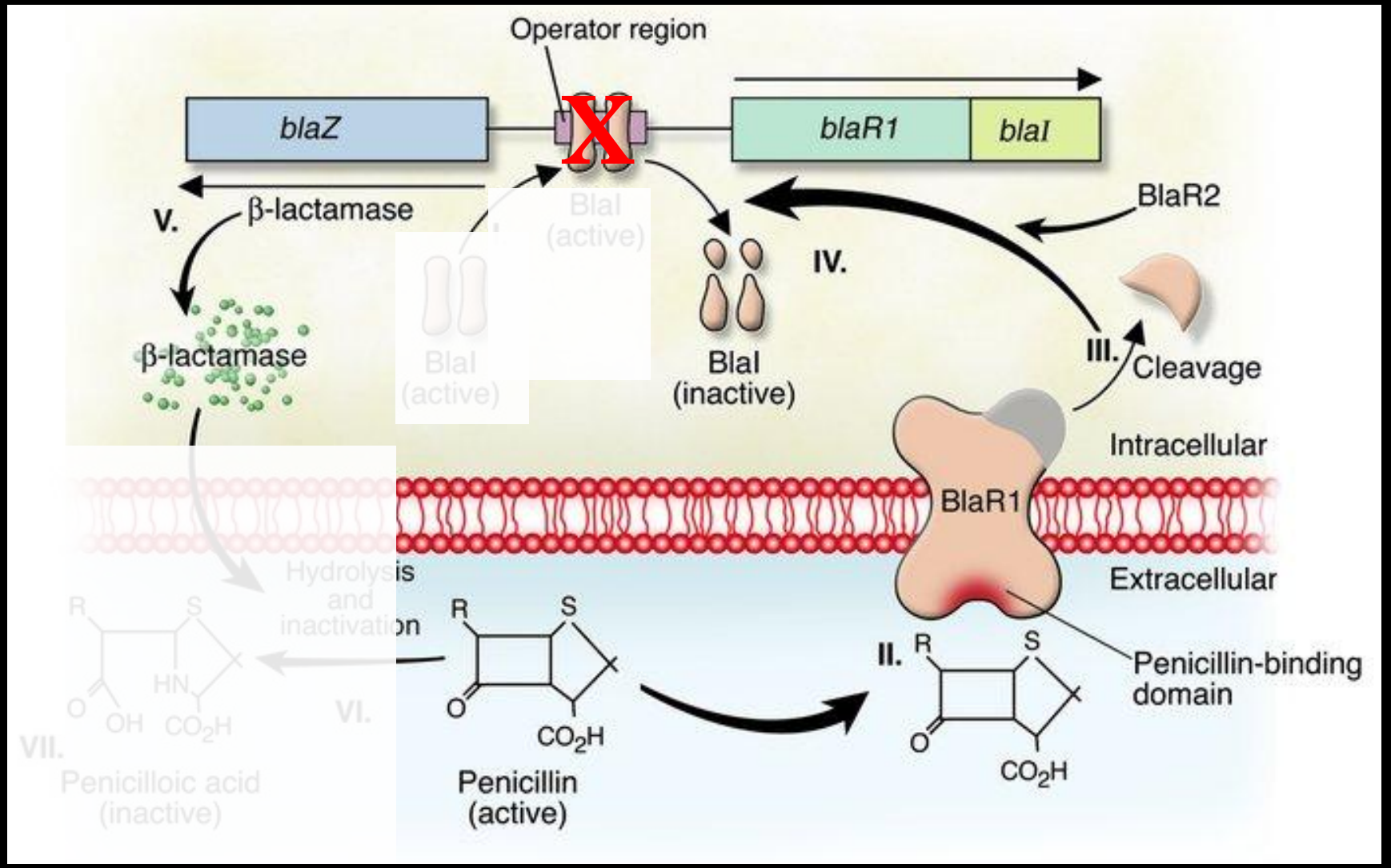
[cracking sound ensues]



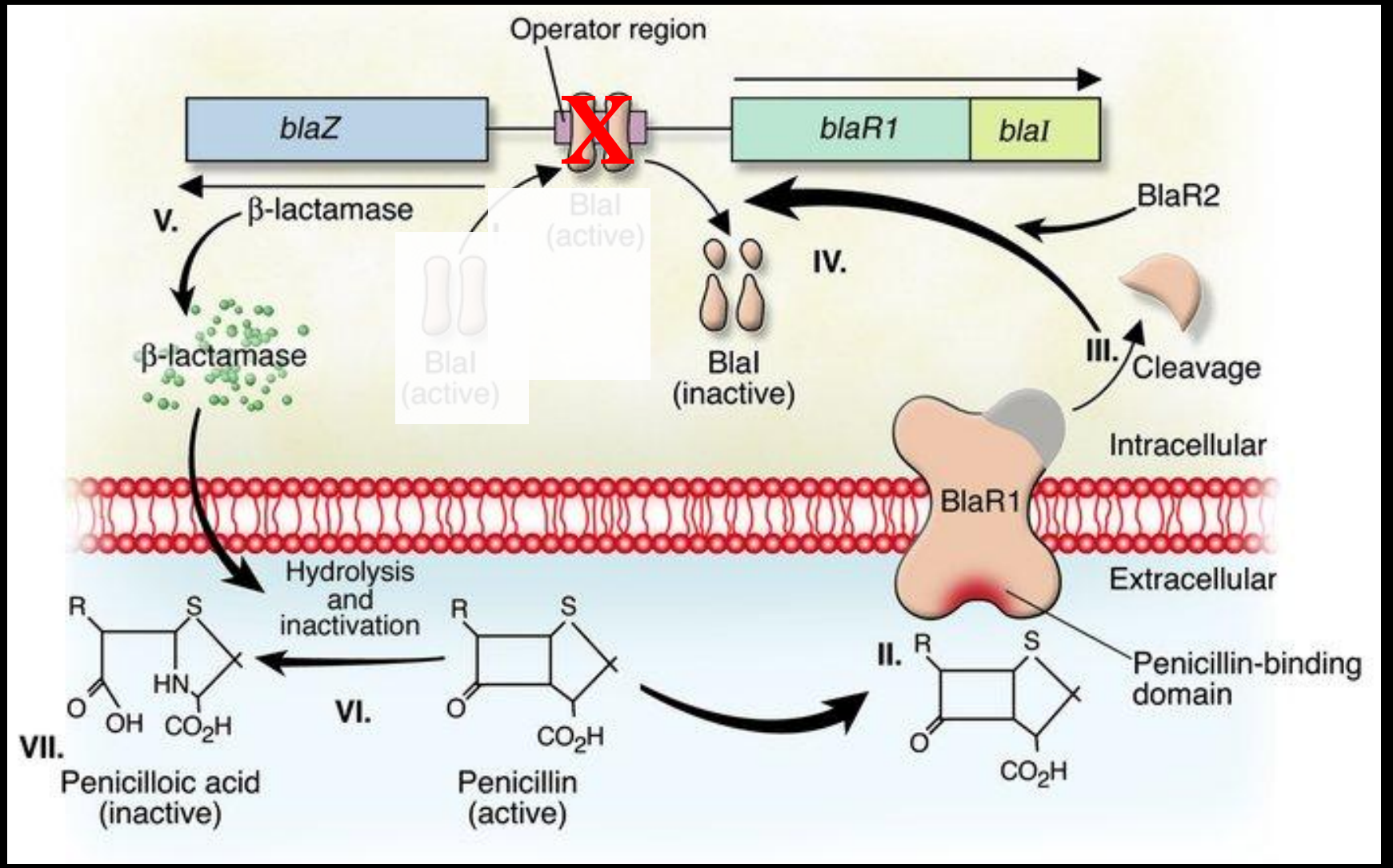
Destroy the repressor, activate the system



Transcribe and translate - the beginning of the end

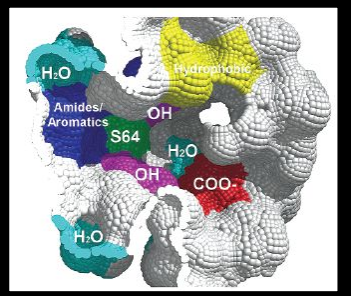


Export and destroy

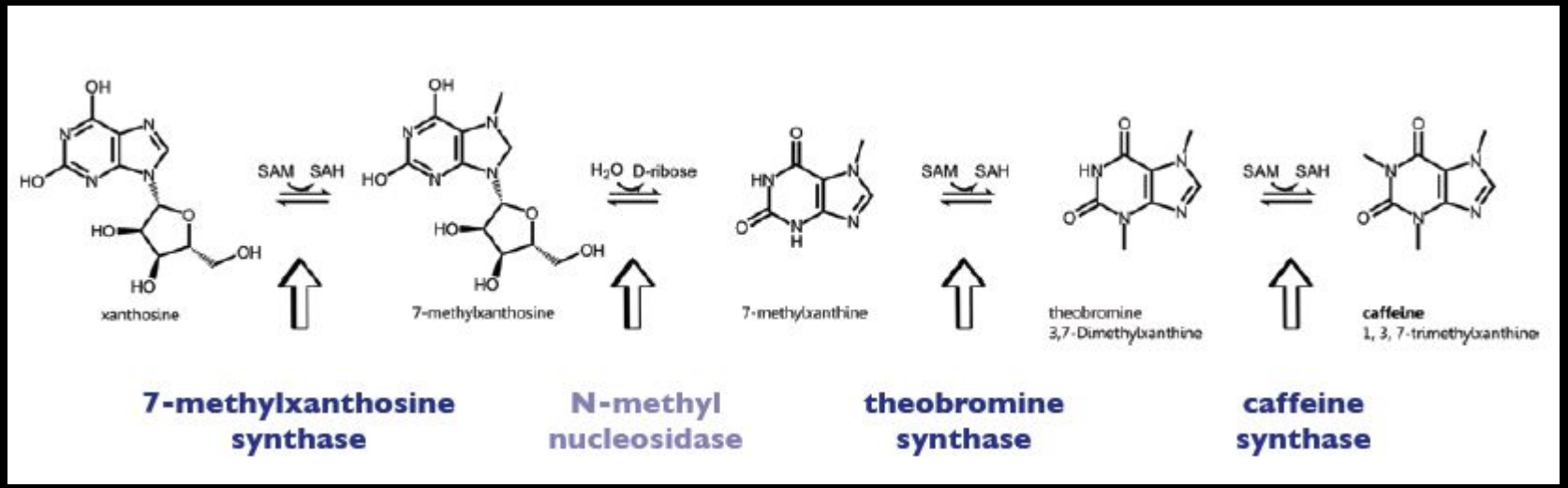


So

- Pathways are important but can be fragile
- Different parts of a protein can play different roles
  - Binding small molecules such as antibiotics, food / energy molecules, etc.
  - Contact with other proteins or DNA
  - Assembly
  - Internal stability once assembled



- These functions impose different *constraints* on protein sequence and structure, and affect how gene and protein sequences can evolve
  - *'Ultraconserved' elements*



http://parts.igem.org/Part:BBa_K801077