Laatste college => Tentamen

- NB: Boek is leidend, het kan zijn dat ik iets niet besproken heb wat wel in het boek staat!
- Bijv. weten welk virus welk replicatiemechanisme hanteert en wat uniek is.
- Bijv. de activiteit van de besproken AB toxines in detail.
- Bijv. activiteit van antibiotica weten in detail…

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Schema Micro2

Les	Hoofdstuk	Paragraaf
1	7	7.1, 7.2, 7.3, 7.8
2	7	7.5, 7.6, 7.7
3	7	7.9, 7.10, 7.11
4	7	7.12, 7.13, 7.14, 7.15
5	5	5.1, 5.2, 5.3, 5.4, 5.5, 5.6
6	5 en 11	5.7, 5.8, 11.1, 11.2
7	11	11.6, 11.7, 11.8 (MS2 niet)
8	11	11.9, 11.11,
9	11	11.13, 11.15, 11.16
10	24	24.1, 24.2, 24.5
11	25	25.1, 25.2, 25.3, 25.5
12	25	25.6, 25.7, 25.8
13	28 en 8	28.5, 28.6, 28.7, 8.11
14	Oefententamen	Alles

NB: Hfdstnrs niet accuraat

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CHAPTER 28

Clinical Microbiology and Immunology

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Infectie

Schimmel:

Penicillium chrysogenum

NB: Observeer zonder verwachtingen te integreren.

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Serendipiteit

The Nobel Prize
Physiology/Medicine 1945

Sir Alexander Fleming
1881 - 1955

Sir Howard Walter Florey
1898 - 1968

Ernst Boris Chain
1906 - 1979

Alexander Fleming discovered the antimicrobial properties of penicillin in 1928. Twelve years later, Howard Florey and Ernst Chain developed the processes to produce penicillin in sufficient quantity for it to become widely available

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28.5 Antimicrobial Drugs

- Antibiotics are naturally occurring antimicrobial compounds that are produced by fungi or *Bacteria*.
- Needs to have selective toxicity (target pathogens not host cells and tissues).
- Antibiotics are classified by their mechanism of action (different targets)
- Susceptibility of microbes to different antibiotics varies greatly.
  - Gram-positive and gram-negative *Bacteria* vary in their sensitivity to antibiotics.
  - *Broad-spectrum* antibiotics are effective against both groups of *Bacteria*.

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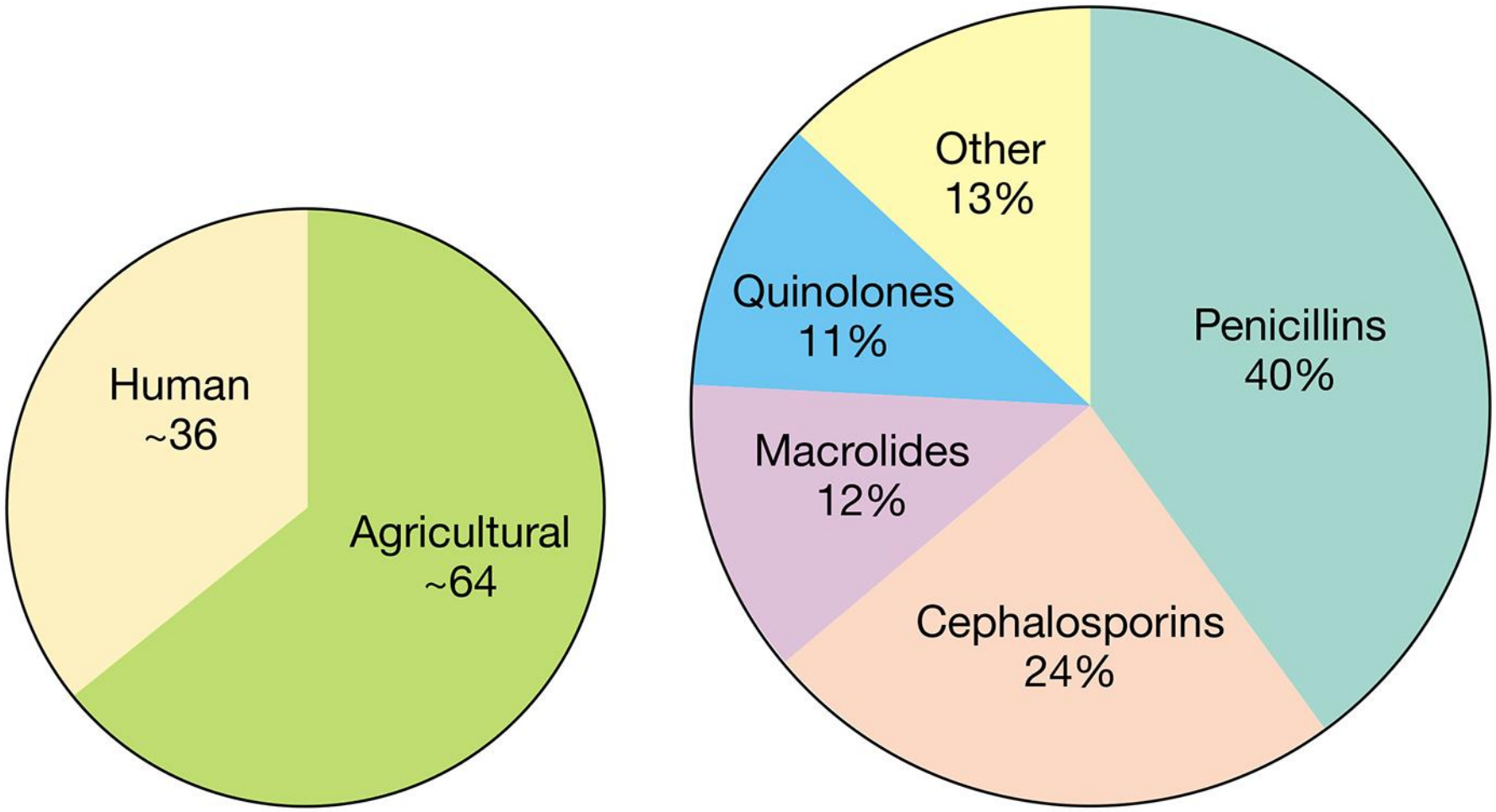
Annual use/production



(a) **Antibiotic usage in metric tons**

(b) **Frequency of antibiotic usage**

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Figure 28.13

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28.5 Antimicrobial Drugs

- The cell wall as a drug target
  - Most of the over 100,000 metric tons of antibiotics used worldwide each year are *β-lactam antibiotics*. (Figure 28.13 A, B)
  - The β-lactam ring is found in penicillins and *cephalosporins.* (Figure 28.14)
    - These drugs inhibit cell wall synthesis (bind transpeptidases irreversibly, hinders crosslinking peptidoglycans)

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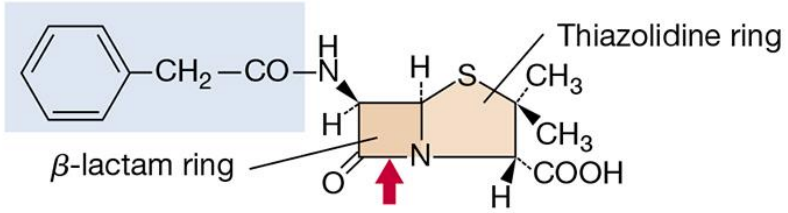
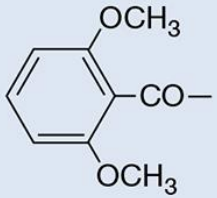
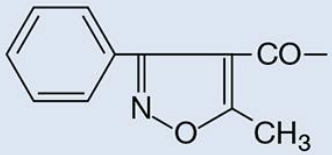
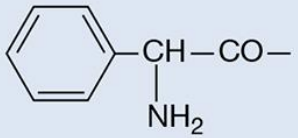
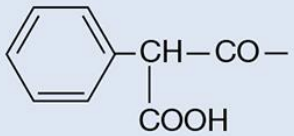
Natural penicillin (penicillin G)
Gram-positive activity; β-lactamase and acid-sensitive



N-acyl group modification	Semisynthetic penicillins
	**Methicillin** acid-stable, β-lactamase-resistant
	**Oxacillin** acid-stable, β-lactamase-resistant
	**Ampicillin** broadened spectrum of activity (especially against gram-negative *Bacteria*), acid-stable, β-lactamase-sensitive
	**Carbenicillin** broadened spectrum of activity (especially against *Pseudomonas aeruginosa*), acid-stable but ineffective orally, β-lactamase-sensitive

- Bind irreversibly to transpeptidases, hinders cross linking peptidoglycans
- Discriminative?
- Hinders all bacterial cell wall formation?

Figure 28.14

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28.5 Antimicrobial Drugs

- Protein synthesis as a drug target
  - Most of the antibiotics targeting protein synthesis will target translation by binding to the bacterial ribosome.
    - Bacterial ribosomes are slightly different than eukaryotic ribosomes, giving (more or less) selective toxicity.

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		Eukaryotic[4]	Bacterial[4]
Ribosome	Sedimentation coefficient	80 S	70 S
	Molecular mass	~3.2×10^6 Da	~2.0×10^6 Da
	Diameter	~250-300 Å	~200 Å
Large subunit	Sedimentation coefficient	60 S	50 S
	Molecular mass	~2.0×10^6 Da	~1.3×10^6 Da
	Proteins	47	33
	rRNAs	• 28 S rRNA (3354 nucleotides) • 5 S rRNA (120 nucleotides) • 5.8 S rRNA (154 nucleotides)	• 23S rRNA (2839 nucleotides) • 5S rRNA (122 nucleotides)
Small subunit	Sedimentation coefficient	40 S	30 S
	Molecular mass	~1.2×10^6 Da	~0.7×10^6 Da
	Proteins	32	20
	rRNAs	• 18S rRNA (1753 nucleotides)	• 16S rRNA (1504 nucleotides)

https://en.wikipedia.org/wiki/Eukaryotic_ribosome_(80S)

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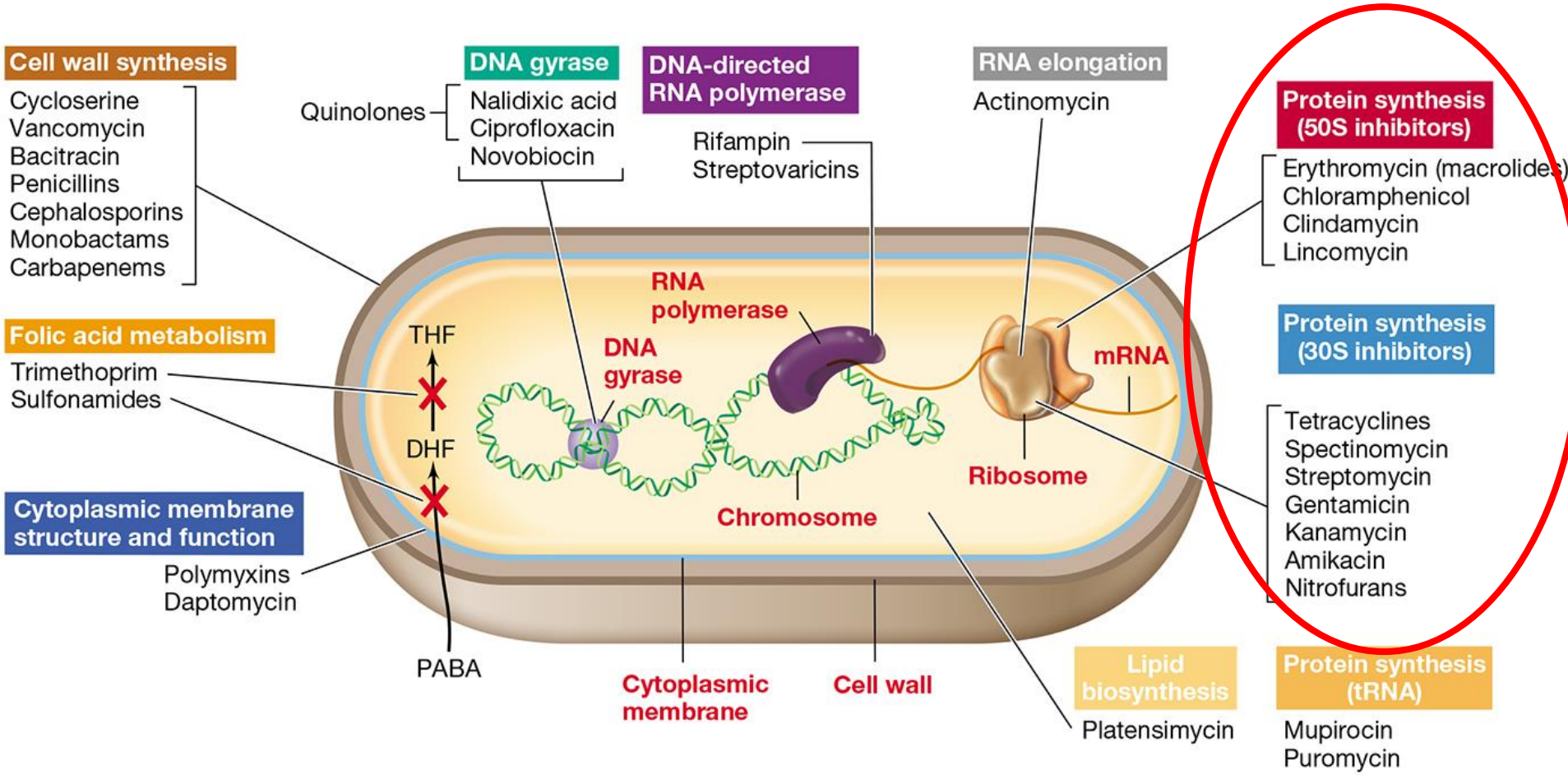

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Figure 28.12

28.5 Antimicrobial Drugs

- Protein synthesis as a drug target
  - Examples of antibiotics that target protein synthesis include: aminoglycosides (e.g. streptomycin), tetracyclines, and macrolide antibiotics.

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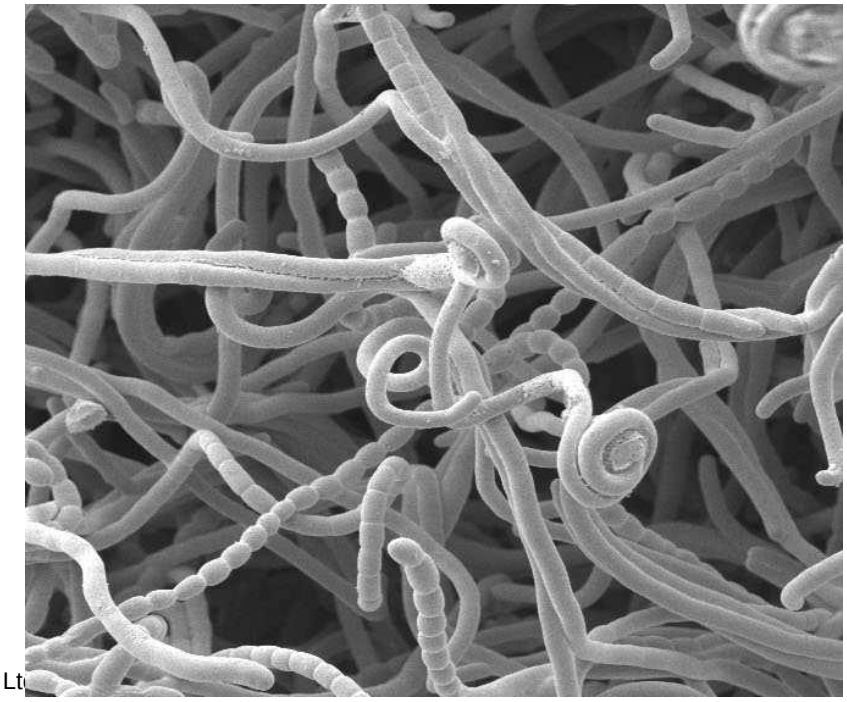
Little bit of history: *Streptomyces*



- Actinomycete
- Filamentous, high GC bacterium
- Enormous source of novel antibiotics ever since the discovery of streptomycin in *S. griseus* in 1943

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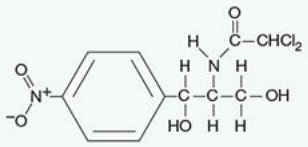
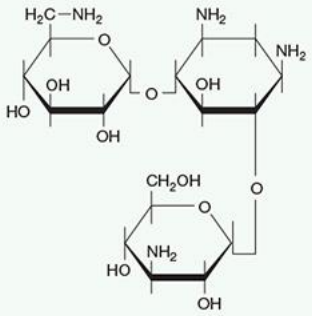
Table 28.4

TABLE 28.5 Selected antibacterial compounds

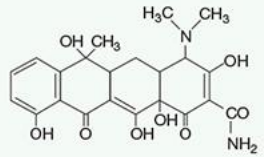
Mode of action	Antibiotic class	Example(s)	Representative structures
Inhibit cell wall synthesis	β-lactams	Penicillins, cephalosporins	Ceftriaxone
	Isoniazids	Isoniazid	Isoniazid
	Polypeptide antibiotics	Vancomycin, bacitracin	Vancomycin (see Figure 28.34)
Inhibit protein synthesis	Aminoglycosides	Streptomycin, kanamycin, gentamicin	Kanamycin
	Tetracyclines	Tetracycline, doxycycline	Tetracycline
	Macrolides	Erythromycin, azithromycin	Erythromycin
	Chloramphenicol	Chloramphenicol	Chloramphenicol



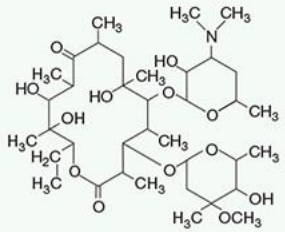
Chloramphenicol



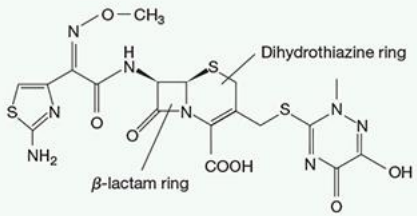
Kanamycin



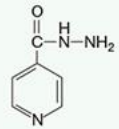
Tetracycline



Erythromycin



Ceftriaxone



Isoniazid

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28.5 Antimicrobial Drugs

- Nucleic acid synthesis as a drug target
  - Quinolones are antibacterial compounds that interfere with DNA gyrase. (*e.g.*, ciprofloxacin)
  - Prevents supercoiling and packaging of DNA.

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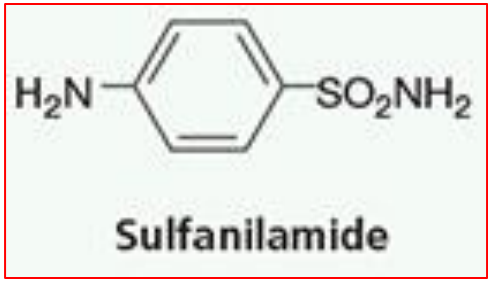
28.5 Antimicrobial Drugs

- Other antibacterial drug targets
  - Growth factor analogs are structurally similar to growth factors but do not function in the cell.
    - analogs similar to vitamins, amino acids, and other compounds
    - Sulfa drugs: PABA analog; blocs nucleic acid synthesis.
  - Isoniazid is a growth analog effective only against *Mycobacterium.*
    - interferes with synthesis of *mycol*ic acid (cell wall)
  - *daptomycin*
    - used to treat gram-positive bacterial infections
    - forms pores in cytoplasmic membrane
  - *platensimycin*
    - new structural class of antibiotic
    - broad-spectrum; effective against MRSA and vancomycin-resistant enterococci



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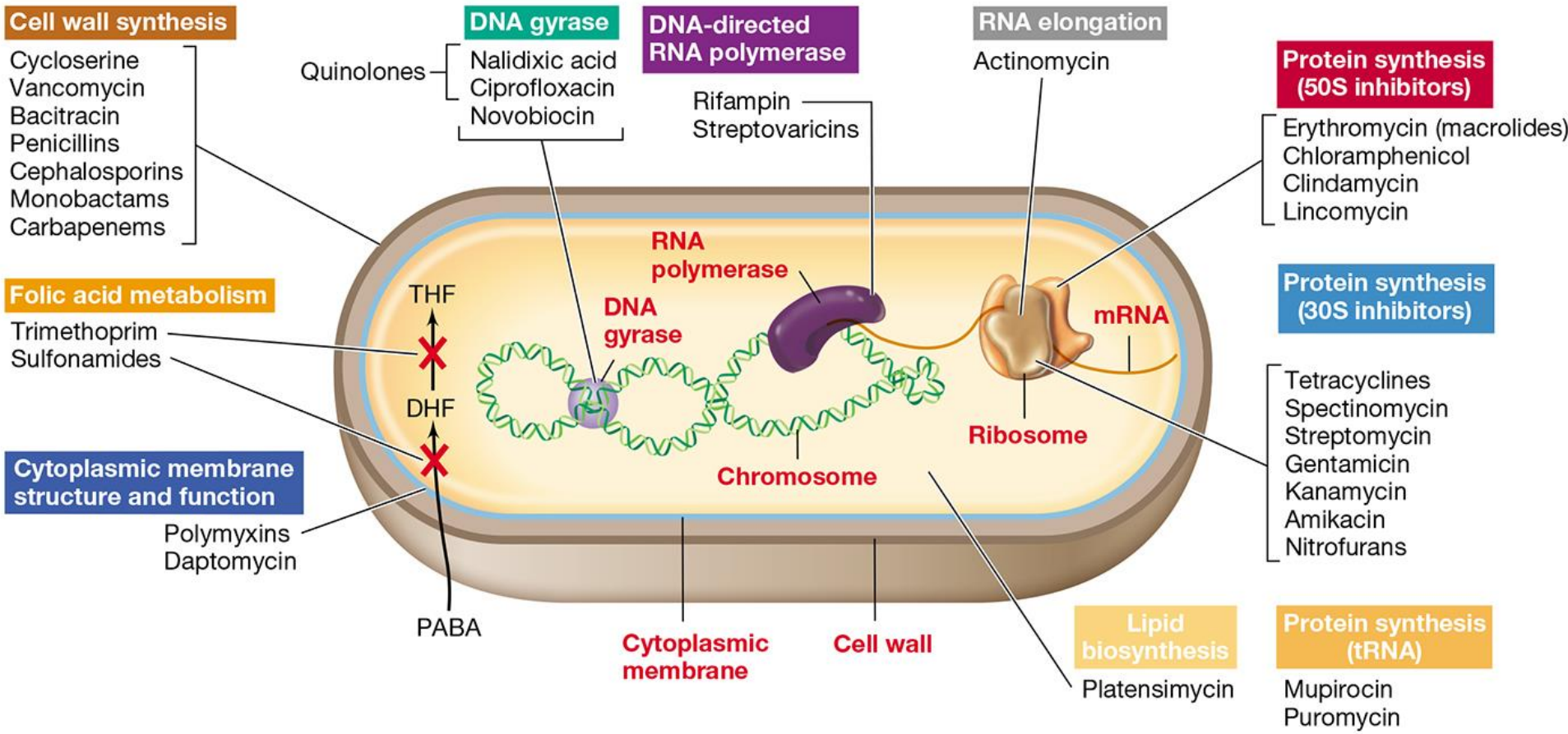

Figure 28.12

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28.6 Antimicrobial Drugs That Target Nonbacterial Pathogens

- Antiviral drugs
  - Most antiviral drugs also target host structures, resulting in toxicity. (Table 28.6)
  - Most successful and commonly used antivirals are the *nucleoside analogs*. (*e.g.,* AZT)
    - block reverse transcriptase and production of viral DNA
    - also called nucleoside reverse transcriptase inhibitors (NRTIs)

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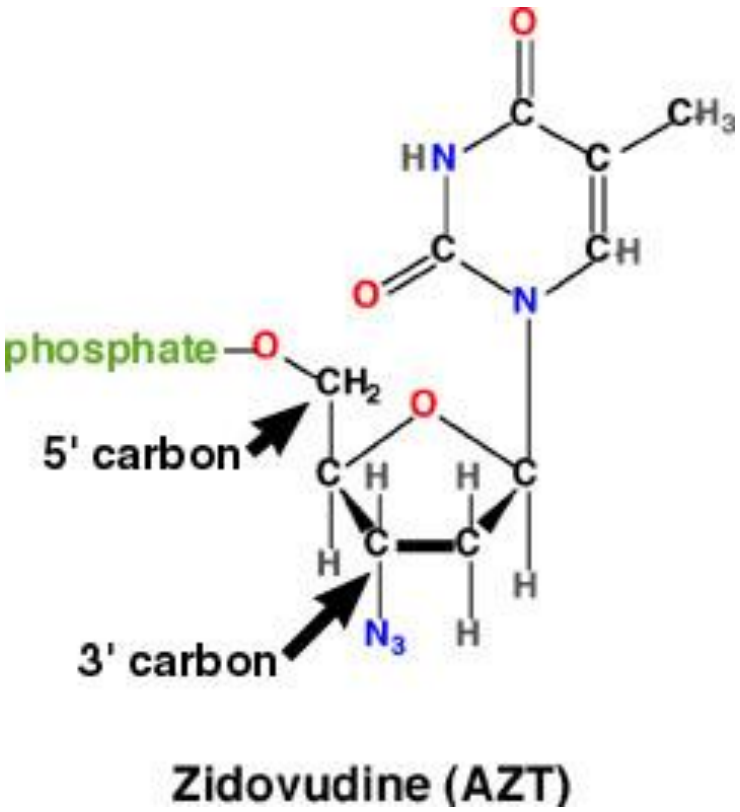
New nucleotides added at 3’-OH



Zidovudine (AZT)

- Retrovir
- Functions as T-analog for reverse transcriptase

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- Remdesivir

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28.6 Antimicrobial Drugs That Target Nonbacterial Pathogens

- Antiviral drugs
  - Nonnucleoside reverse transcriptase inhibitors (NNRTIs) bind directly to RT and inhibit reverse transcription.
    - Protease inhibitors inhibit the processing of large viral proteins into individual components.
    - Fusion inhibitors prevent viruses from successfully fusing with the host cell.

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Class VI: Retrovirus (e.g. HIV)

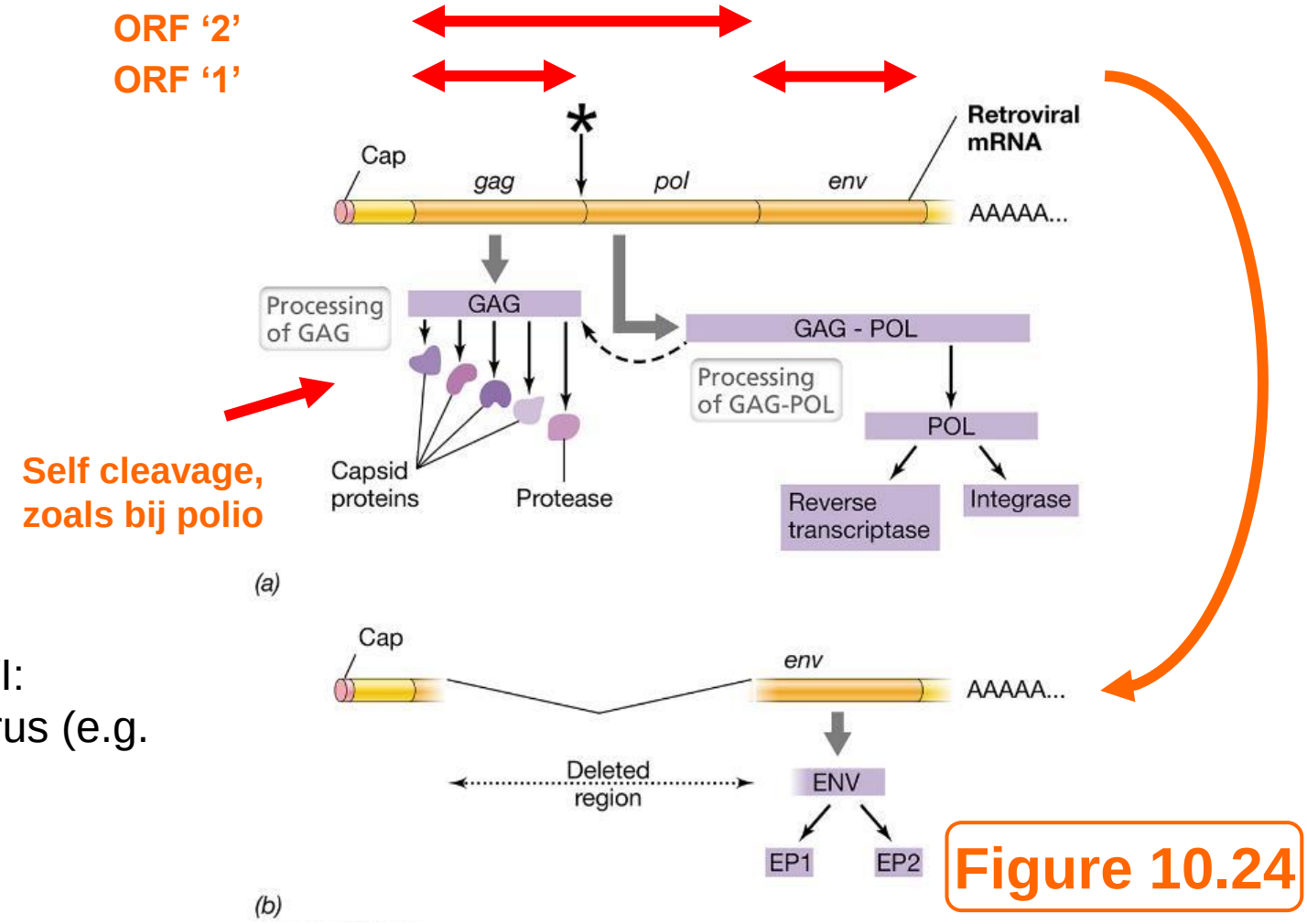

Figure 10.24

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28.6 Antimicrobial Drugs That Target Nonbacterial Pathogens

- Antiviral drugs
  - *Neuraminidase inhibitors* (*e.g.,* Tamiflu) successfully limit influenza infection.
  - *Interferons:* small proteins that prevent viral multiplication by stimulating antiviral proteins in uninfected cells.

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28.6 Antimicrobial Drugs That Target Nonbacterial Pathogens

- Drugs that target eukaryotic pathogens
  - Fungi pose special problems for chemotherapy because they are eukaryotic. (Figure 28.32)
    - Much of the cellular machinery is the same as that of animals and humans.
    - As a result, many antifungals are for topical use.

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TABLE 28.7 Antifungal agents

Category	*Target*	*Examples*	*Use*
Allylamines	Ergosterol synthesis	Terbinafine	Oral, topical
Aromatic antibiotic	Mitosis inhibitor	Griseofulvin	Oral
Azoles	Ergosterol synthesis	Clotrimazole	Topical
		Fluconazole	Oral
		Miconazole	Topical
Chitin synthesis inhibitor	Chitin synthesis	Nikkomycin Z	Experimental
Echinocandins	Cell wall synthesis	Caspofungin	Intravenous
Nucleic acid analogs	DNA synthesis	5-Fluorocytosine	Oral
Polyenes	Ergosterol synthesis	Amphotericin B	Oral, intravenous
		Nystatin	Oral, topical
Polyoxins	Chitin synthesis	Polyoxin A and B	Agricultural

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Table 28.6

28.6 Antimicrobial Drugs That Target Nonbacterial Pathogens

- A few drugs target unique metabolic processes unique to fungi, such as cell wall synthesis. (Table 28.6)
  - *Ergosterol inhibitors* target the unique fungal plasma membrane component ergosterol.
  - *Echinocandins* inhibit 1,3 β-D glucan synthase and are used to treat *Candida* infections.
- Other drugs target chitin biosynthesis, target folate biosynthesis, or disrupt microtubule aggregation.
- Like antibiotic resistance, antifungal resistance is on the rise.

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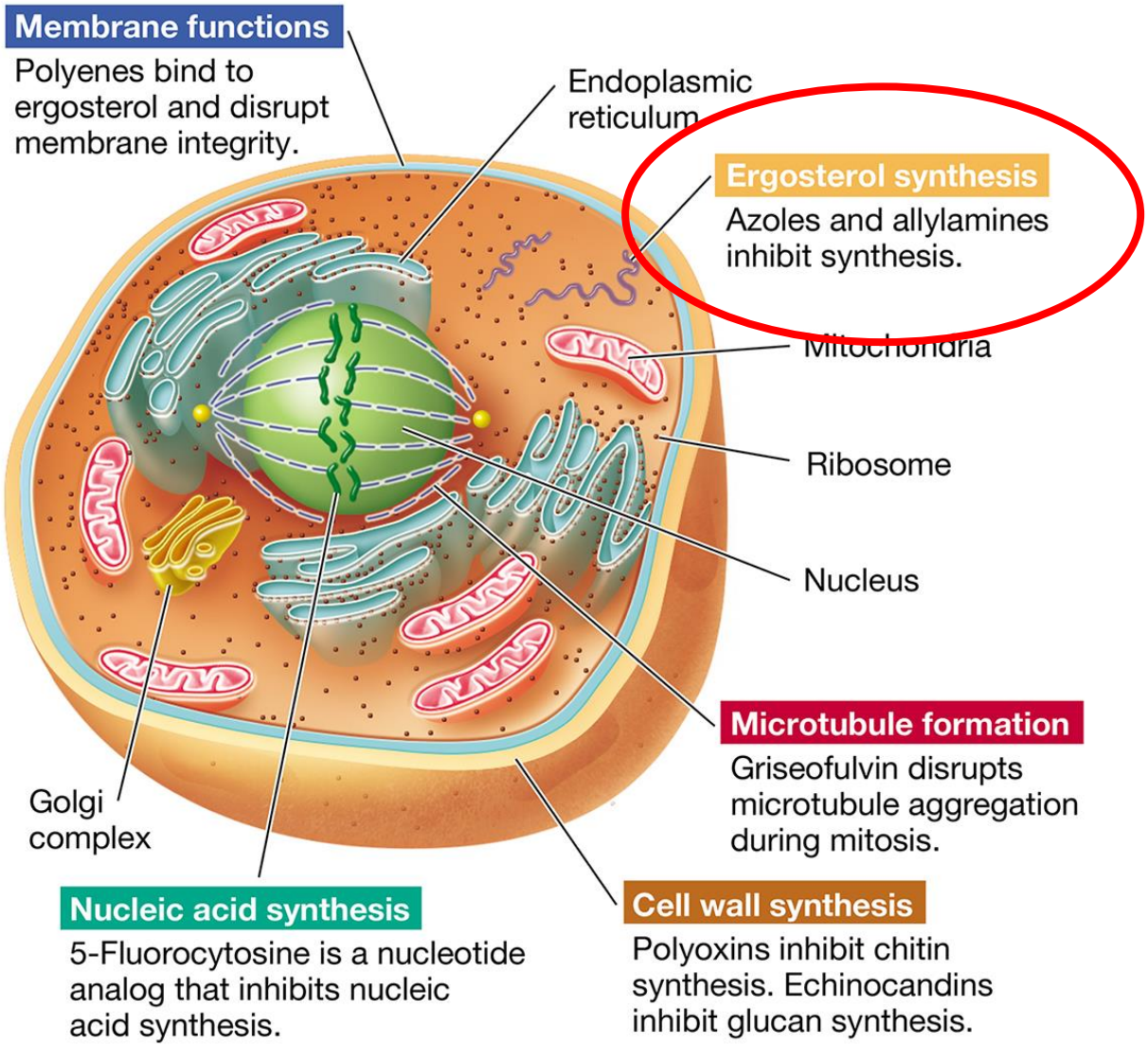

Figure 28.17

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Microbiologie 2: Les 13
Antibiotic resistance

28.12 Antimicrobial Drug Resistance and New Treatment Strategies

- Antimicrobial drug resistance
  - the *acquired* ability of a microorganism to resist the effects of a chemotherapeutic agent to which it is normally sensitive
  - at least five reasons that microorganisms are naturally resistant to certain antibiotics (Table 28.8)
    - Organism is impermeable to antibiotic.
    - Organism can inactivate the antibiotic.
    - Organism may modify the target of the antibiotic.
    - Organism may develop a resistant biochemical pathway.
    - Organism may be able to pump out the antibiotic (*efflux*).

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TABLE 28.8 Bacterial resistance to antibiotics

Resistance mechanism	*Antibiotic example*	*Genetic basis of resistance*	*Mechanism present in*
Reduced permeability	Penicillins	Chromosomal	Gram-negative bacteria
Inactivation of antibiotic Examples: b-lactamases; modifying enzymes, such as methylases, acetylases, phosphorylases, and others	Penicillins	Plasmid and chromosomal	*Staphylococcus aureus* Enteric bacteria
	Chloramphenicol	Plasmid and chromosomal	*Neisseria gonorrhoeae* *Staphylococcus aureus*
	Aminoglycosides	Plasmid	Enteric bacteria
Alteration of target Examples: RNA polymerase, rifamycin; ribosome, erythromycin and streptomycin; DNA gyrase, quinolones	Erythromycin Rifamycin Streptomycin Norfloxacin	Chromosomal	*Staphylococcus aureus* Enteric bacteria Enteric bacteria Enteric bacteria *Staphylococcus aureus*
Development of resistant biochemical pathway	Sulfonamides	Chromosomal	Enteric bacteria *Staphylococcus aureus*
Efflux (pumping out of cell)	Tetracyclines Chloramphenicol	Plasmid Chromosomal	Enteric bacteria *Staphylococcus aureus* *Bacillus subtilis*
	Erythromycin	Chromosomal	*Staphylococcus*

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Table 28.8

8.11 Antibiotic Targets and Antibiotic Resistance

- Antibiotic resistance: efflux pumps and metabolic bypasses
  - *Efflux pumps* are ubiquitous and transport various molecules, including antibiotics, out of the cell.
    - lowers intracellular concentration, allowing cell to survive at higher external concentrations
    - Many act promiscuously and transport different classes outside cell.
    - contribute to multidrug resistance (MDR)
    - AcrAB-TolC of *E. coli* is one of the best characterized and pumps out rifampicin, chloramphenicol, fluoroquinolones.
  - Biofilm growth leads to increased resistance.
    - makes infections difficult to treat
    - AcrAB-TolC efflux pump genes upregulated when cells enter biofilm growth mode
    - *Pseudomonas aeruginosa* encodes several multidrug efflux pumps that are more active when cells grow in an attached state.

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8.11 Antibiotic Targets and Antibiotic Resistance

- Antibiotic resistance: metabolic bypasses
  - Antibiotic target is no longer essential.
    - example: Methicillin-resistant *Staphylococcus aureus* (MRSA)
      - Methicillin is a β-lactam resistant to β-lactamase cleavage.
      - MRSA strains contain a DNA island called *Staphylococcus chromosomal cassette for methicillin resistance (SCCmec)* that encodes MecA, an *alternative* penicillin-binding protein that is not recognized by β-lactams.
      - MRSA synthesize MecA only in the presence of β-lactams due to repressor MecI and β-lactam sensor MecR1.

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28.7 Antimicrobial Drug Resistance and New Treatment Strategies

- Antimicrobial drug resistance
  - Because antibiotic-resistant genes exist in nearly every population, physicians cannot use the same antibiotic for long. (Figure 28.18)
  - Any use of antibiotics selects for resistance, increasing the number of resistant bacteria in any bacterial population.
    - Overuse accelerates this process.

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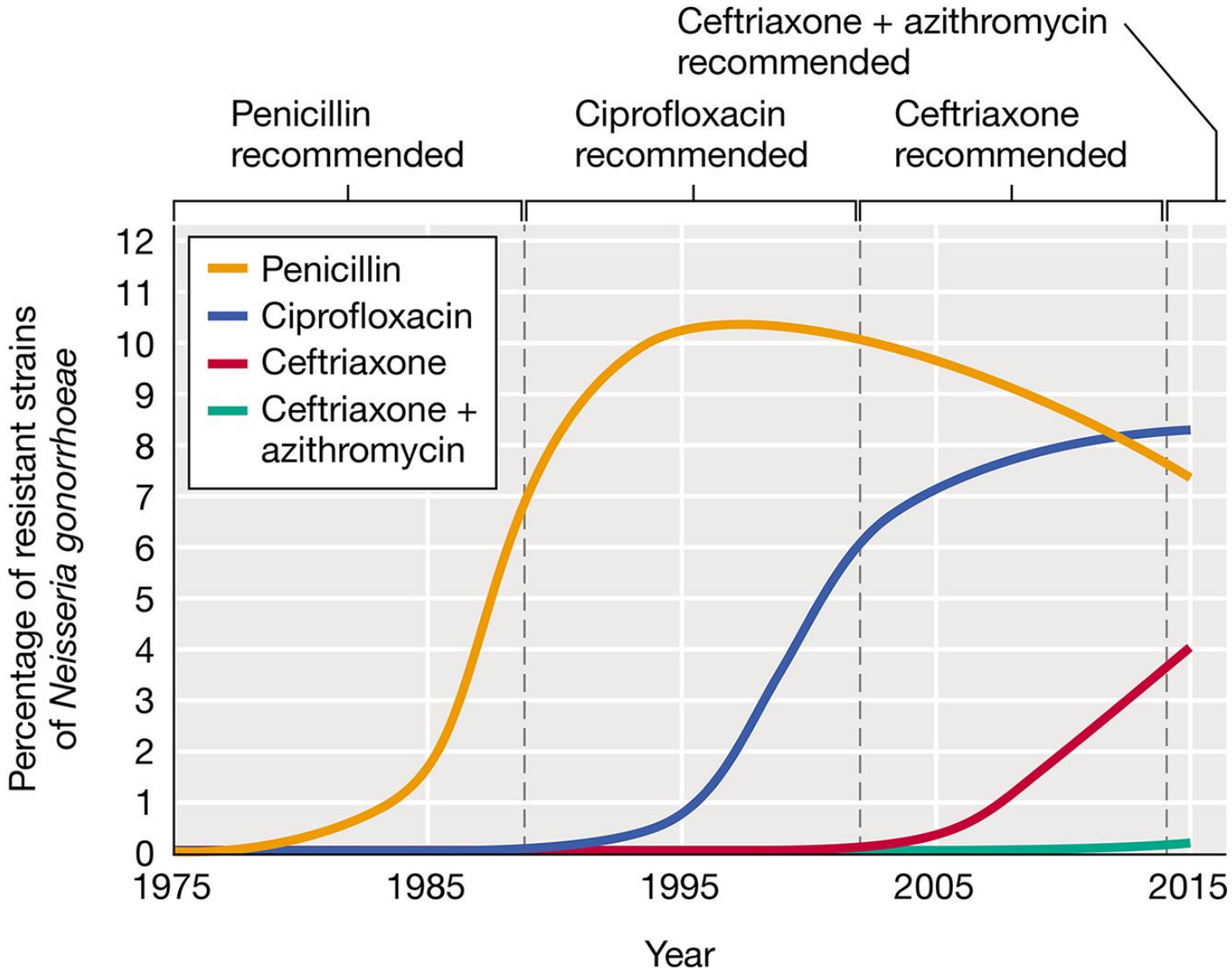

Figure 28.18

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28.7 Antimicrobial Drug Resistance and New Treatment Strategies

- Antimicrobial drug resistance
  - Almost all pathogenic microbes have acquired resistance to some chemotherapeutic agents.
  - A few pathogens have developed resistance to all known antimicrobial agents (MDR).
  - Resistance can be minimized by using antibiotics correctly and only when needed.
  - Resistance to a certain antibiotic can be lost if antibiotic is not used for several years.

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28.7 Antimicrobial Drug Resistance and New Treatment Strategies

- New drugs and new treatment strategies
  - Long-term solution to antimicrobial resistance relies on the development of new antimicrobial compounds.
  - Modification of current antimicrobial compounds is often productive, such as the production of vancomycin. (Figure 28.34)
  - Automated chemistry methods (combinatorial chemistry) have sped up drug discovery.

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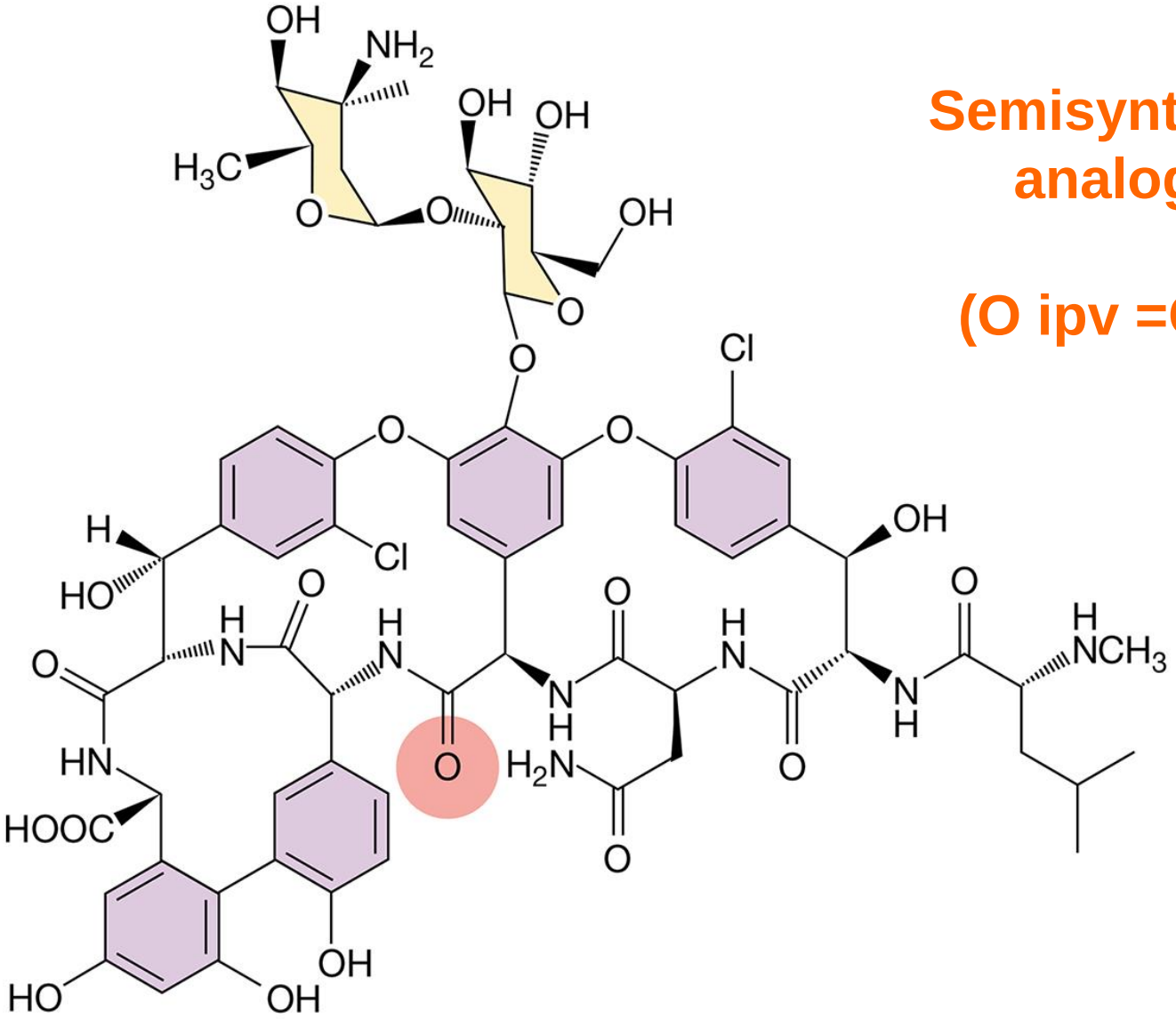

Vancomycin

Semisynthetic analogs

(O ipv =CH_2)

Figure 28.19

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28.7 Antimicrobial Drug Resistance and New Treatment Strategies

- New drugs and new treatment strategies
  - Computers can now be used to design molecules to interact with specific microbial structures.
  - Most successful example is *saquinavir.* (Figure 28.20)
    - binds to active site of HIV protease
  - New methods of screening natural products are being used.
  - led to the discovery of platensimycin from the soil bacterium *Streptomyces platensis*
  - Combinations of drugs with enzyme inhibitors (*e.g.,* clavulanic acid inhibits β-lactamase) is given with ampicillin.
  - Combinations of drugs can be used (*e.g.,* ampicillin and sulbactam).

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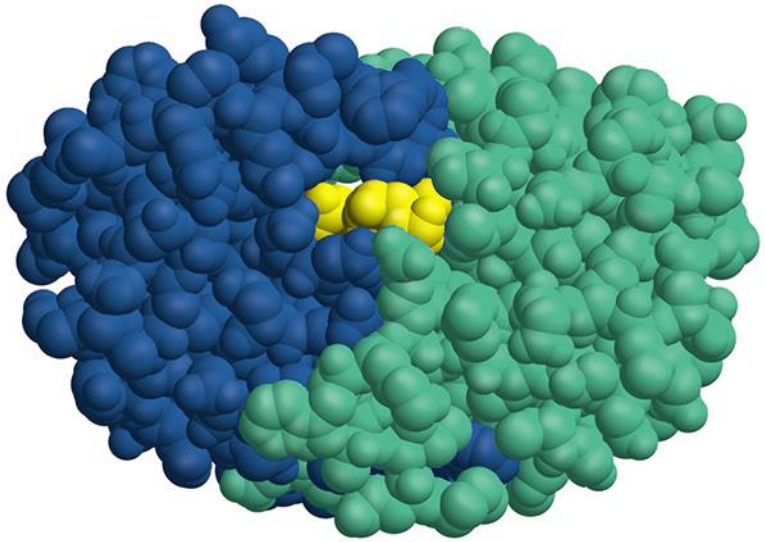
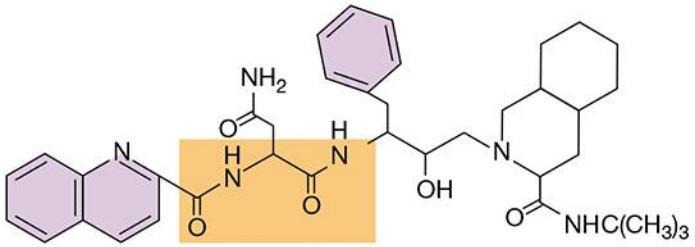
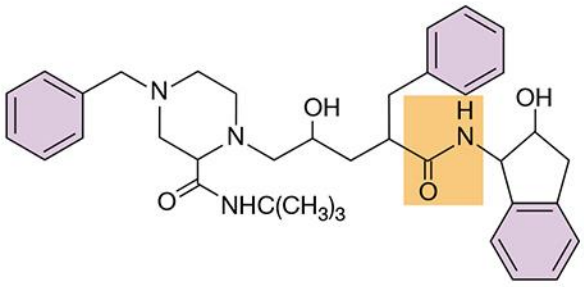


(a) **HIV protease**



Saquinavir



Indinavir

(b)

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Figure 28.20

EINDE LES 13

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CHAPTER 8

Molecular Biology of Microbial Growth

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8.11 Antibiotic Targets and Antibiotic Resistance

- Antibiotics are antimicrobials naturally produced by microbes.
  - kill or inhibit bacterial growth
  - target essential molecular processes

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8.11 Antibiotic Targets and Antibiotic Resistance

- Antibiotics that target major molecular processes
  - Many antibiotics target DNA replication, RNA synthesis, and translation. (Figure 7.21*a*)
    - Quinolones target DNA gyrase and topoisomerase IV by interfering with DNA unwinding and replication.
    - Rifampin and actinomycin prevent RNA synthesis by blocking RNA polyermase active site or RNA elongation.
  - inhibition of protein synthesis
  - Ribosomes in *Bacteria* are 70S; eukaryotic are 80S.
    - Puromycin binds to A site in 70S ribosome, inducing chain termination and inhibition protein synthesis.
    - Aminoglycoside antibiotics (*e.g.,* streptomycin) target 16S rRNA of 30S ribosome, leading to error-filled proteins.

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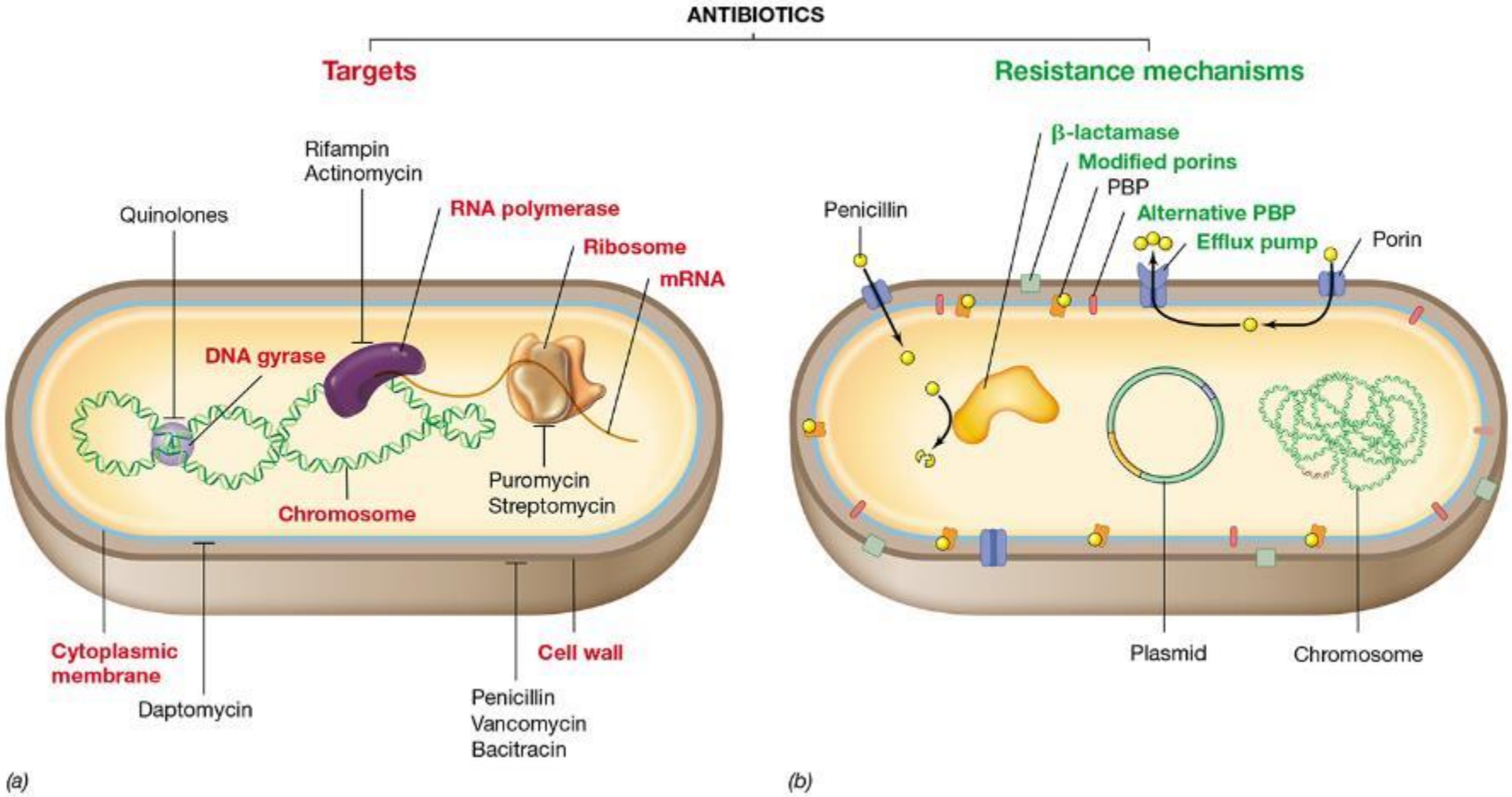

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Figure 8.27/28

8.11 Antibiotic Targets and Antibiotic Resistance

- Antibiotics that target the cell membrane and wall
  - Daptomycin specifically binds to phosphatidylglycerol residues of bacterial plasmid membrane, leading to pore formation, depolarization, and death.
  - Polymyxins are cyclic peptides whose long tails target LPS layer, disrupting membrane and causing leakage and death
  - targeting peptidoglycan synthesis
    - β-lactams (penicillin, cephalosporin, derivatives) interfere with transpeptidation (formation of cross-links)
    - Vancomycin binds to pentapeptide precursor and prevents interbridge formation.
    - Bacitracin binds to bactoprenol and prevents new peptidoglycan precursors from reaching site of synthesis.

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8.11 Antibiotic Targets and Antibiotic Resistance

- Antibiotic resistance: spontaneous mutations and antibiotic modification
  - resistance mechanisms genetically encoded in four classes: modification of drug target, enzymatic inactivation, removal via efflux pumps, metabolic bypasses
  - Random chromosomal mutations can lead to resistance.
    - Example: Spontaneous mutants resistant to antibiotic rifampin can be obtained by exposing a large population.
  - Resistance genes can exist on mobile genetic elements and be transferred by horizontal gene flow.
    - Many mobile resistance genes encode enzymes that inactivate antibiotic (*e.g.,* β-lactamase cleaves a ring structure; an acetylating enzyme adds acetyl groups to chloramphenicol).

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