Microbiology

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Any suggestions for improvements would be greatly appreciated.

...There are bred certain minute creatures which cannot be seen by the eyes, which float in the air and enter the body through the mouth and nose and there cause serious diseases... A well-ventilated place is more easily cleared if anything offensive is brought in. Furthermore, being exposed to the sun during the whole day, it is more wholesome, as any small creatures which are bred nearby and brought in are either blown away or quickly die from the lack of humidity.

–Marcus Terentius Varro, *On Agriculture* I, XII (ca. 50 B.C.)

In racks were rows of test-tubes, each bearing a neatly-written label, and there were files of specimen slides near the big microscope... 'Petrie found, in the blood of a patient... a sort of hybrid germ, which I lack the knowledge to describe to you. He found Sleeping sickness and Plague combined...'

Petrie lay in the shadow of his working-bench... I saw that the apparently rigid fingers grasped a hypodermic syringe. Near to his upraised hand was a vessel containing a small quantity of some milky fluid... 'A preparation of his own–to which I have heard him refer as "654." He believed it was a remedy...'

–Sax Rohmer, *The Bride of Fu Manchu* (1933)

Overview

Microbiology covers different categories of microorganisms, most of which include important pathogenic and nonpathogenic examples. Prions are rogue proteins that need to hijack a cell to reproduce, whereas viruses are rogue genes that need to hijack a cell to reproduce. Bacteria are single simple (prokaryotic) cells. Algae are single-celled (eukaryotic) plants that can use photosynthesis to harness light energy; fungi are fairly similar but lack photosynthesis. Protozoa are simple animals composed of a single eukaryotic cell. Helminths are multi-celled eukaryotic worms that start off like microorganisms but may grow to significant size. For each of these categories of microorganisms, this summary will discuss how the organisms work, how some of them can cause disease, how infections by those pathogenic members can be treated or prevented, and how some microorganisms can be harnessed for useful applications.

Contents

1 General Principles of Microbiology

Microbiology covers all sorts of infectious diseases you never knew existed, from **pathogenic** (disease-causing) individual proteins to parasitic worms, and may leave you curled up reading the *Merck Manual*, trying at all costs to avoid germs or imagining what you already have. It's really cool in a gloriously icky sort of way. Microbiology also includes a variety of non-pathogenic (non-disease-causing) microorganisms, some of which have useful practical applications. As shown in Fig. 1, microbiology integrates information from biochemistry, molecular biology, and cell biology, and applies that to organisms that are mostly single-celled or replicate within individual cells. As such, microbiology is also very important for understanding the health or diseases of multicellular organisms from plants to human beings. This section will give an overview of the categories of microorganisms, the natural defenses they must evade to infect us, and common methods of studying microorganisms in the laboratory.

1.1 Categories of Microorganisms

Grab some hand sanitizer and take a gander at Fig. 2, which shows the categories of microorganisms from smallest to largest in physical size:

(a) **Prions** are just rogue proteins and not full-fledged living, breathing microorganisms, but they can spread from one animal or human host to another and kill their hosts, so we will let them into the club. To replicate, they persuade some of the host's own proteins to change slightly and turn into new prion proteins, like Minecraft junkies recruiting more young Minecraft addicts. Prions are especially fond of Swiss-cheesing brains, making cows mad and cannibals crazy.

(b) **Viruses** are rogue genes that invade host cells, command cells to make more copies of the viral genes, and usually (but not always) kill the host cells on the way out, before spreading to infect more host cells. They include everything from the common cold to Ebola.

(c) **Bacteria** are prokaryotic or primitive cells, but we don't want to hurt their feelings. More respectable bacteria make an independent living in the environment. Some bacteria hang out in the space outside our cells in our bodies, where they may cause disease or even help (as with the bacteria that aid digestion in our intestines). Some of the nastier bacteria actually invade our cells and replicate inside our cells, for example in tuberculosis lung infections.

(d) **Fungi and algae** are eukaryotic (complex) cells similar to our own. Fungal cells are surrounded by a thick cell wall and can cause anything from athlete's foot to mushrooms on pizza. Algae are fairly similar but with a different type of cell wall, and with the ability to convert sunlight to energy via photosynthesis. Algae don't infect much except coral (which they actually help), since it is usually fairly dark inside an animal or human host.

(e) **Protozoa** are eukaryotic cells that are even more similar to our own–they are basically just single-celled animals. Some of the more vicious ones (like malaria) can still invade our own cells though. Other protozoa just spend their whole lives harmlessly floating around in ponds and hoping that biology students with microscopes might someday pay attention to them.

(f) **Helminths** is the intimidating scientific name for worms. Most worms are perfectly happy to dig around in your backyard, but some specific types of worms can invade your body, usually when they are young and microscopic in size, then grow to disgusting size. They include everything from tapeworms to those ear critters in Star Trek's *Wrath of Khan*.

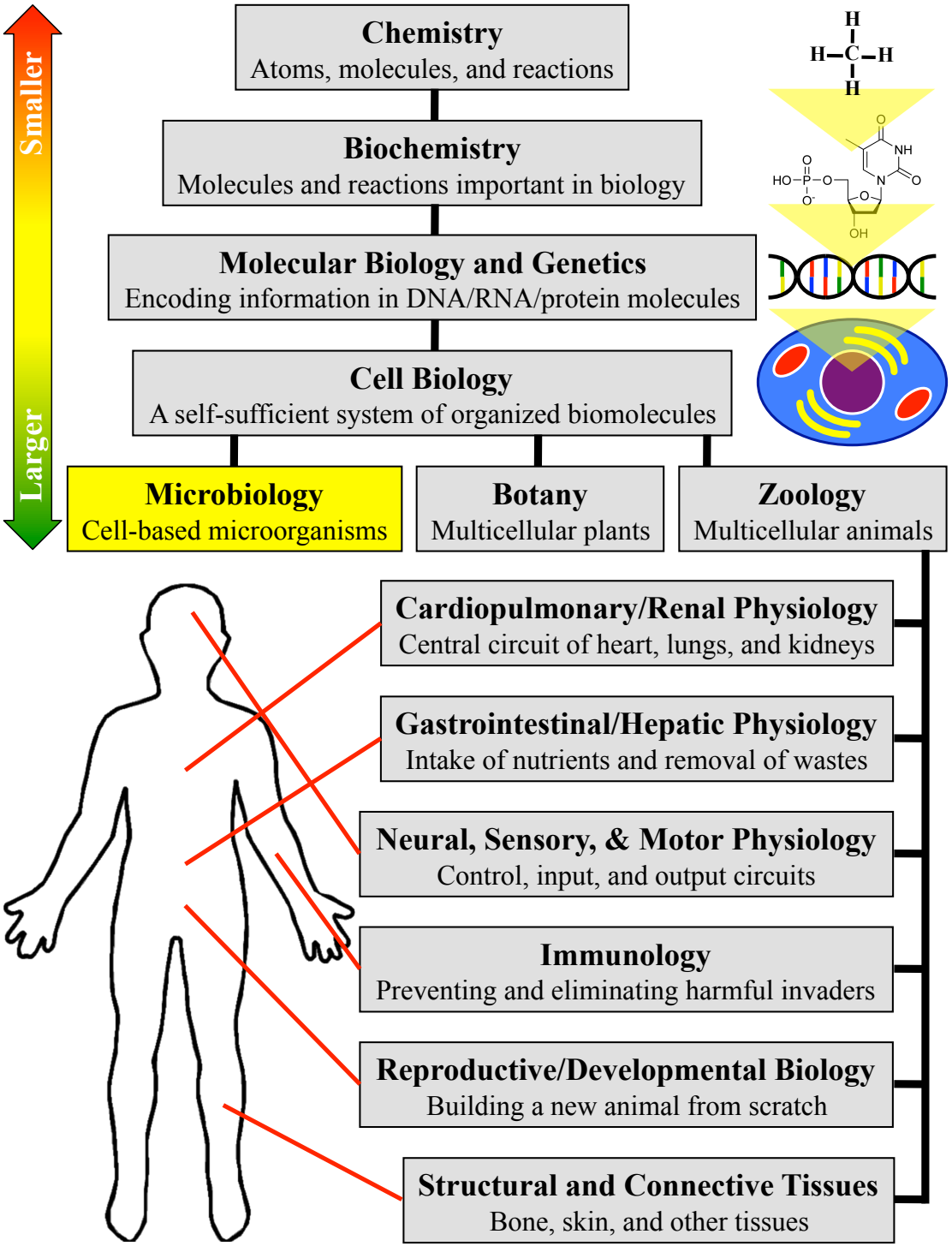


Fig. 1. Relationship between microbiology and other subjects.

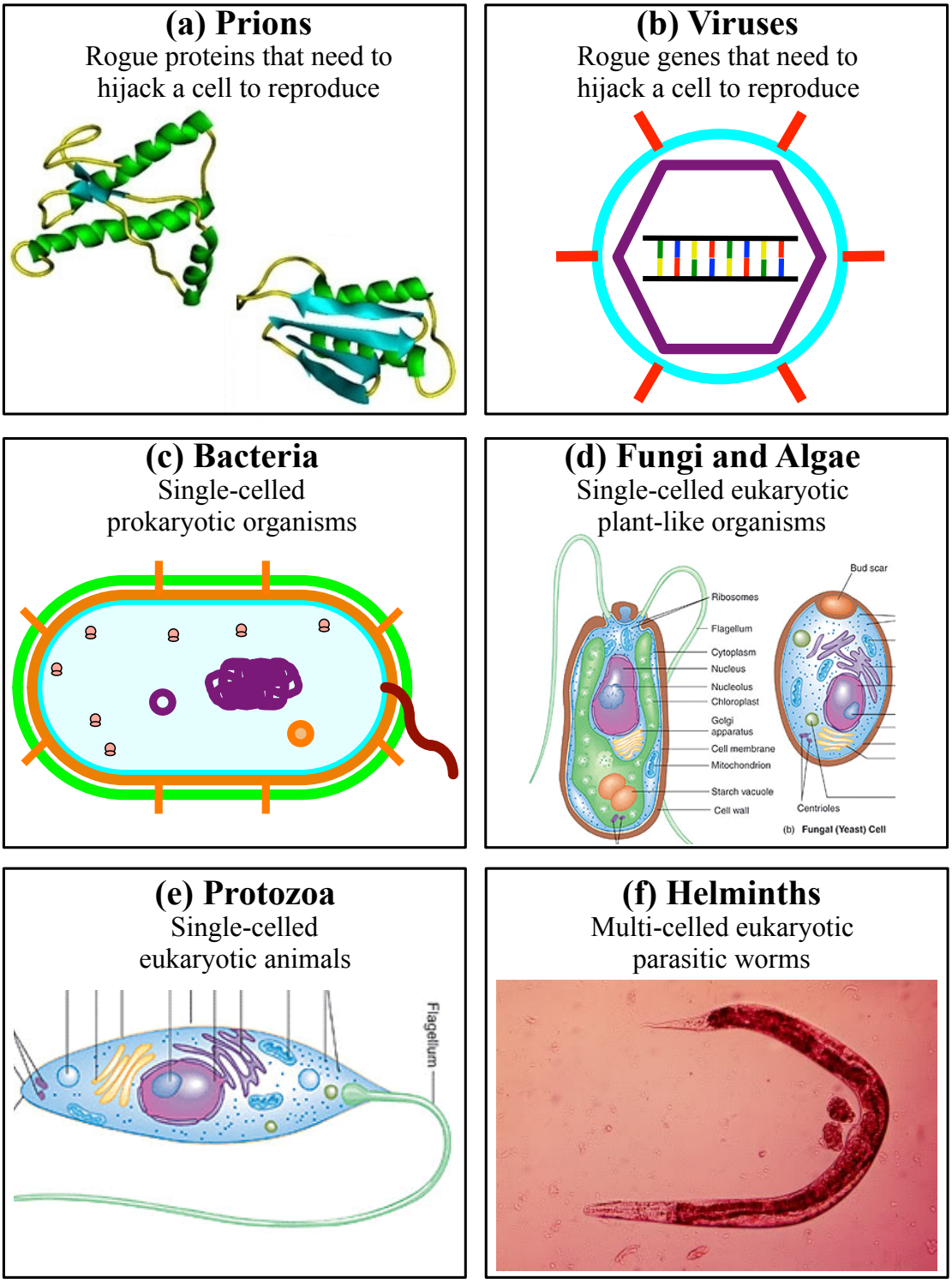


Fig. 2. Categories of microorganisms, from smallest to largest critters.

1.2 Natural Defenses Against Microorganisms

Humans, animals, and even plants have developed natural defenses that prevent, control, or eliminate infections by most microbial pathogens. Those defenses work very well and protect us against all sorts of germs that we don't even notice because we are naturally resistant to them. The microbial pathogens we do notice are the ones that have found devious ways to avoid those natural defenses. Natural defenses in humans and other vertebrate animals fall into two broad categories, intracellular defense pathways that are present inside virtually all of our cells (like a burglar alarm in every house in the city), and extracellular defenses provided by a relatively small number of immune system cells patrolling our body (like police patrolling a city to look for criminals).

Intracellular defenses are covered in some detail in *Cell Biology* section 2, but some of the major intracellular defenses include:

- **The interferon defense pathway** (Fig. 3) uses a variety of sensors to detect different categories of pathogens that might invade cells. If a cell detects those signals and realizes that it is infected, it will produce and send interferon proteins to nearby uninfected cells, like little Paul Reveres telling those nearby cells that the Red Coats are coming and that they need to prepare their defenses. Sneaky pathogens have various ways to block their detection within infected cells, to block the interferon warning signals, and/or to block activated defenses in cells that have received the interferon signals.

- **The inflammatory defense pathway** (Fig. 4) also uses a variety of sensor to detect different categories of pathogens, including pathogens both inside and outside the cells. If a cell detects pathogens inside or outside itself, it activates inflammatory defenses against pathogens inside itself and also produces pro-inflammatory signaling molecules to trigger inflammatory defenses in nearby cells. Smart pathogens have found ways to block the activation or responses of the inflammatory pathway. Some pathogens even deliberately trip the inflammatory alarms and then actually use those predictable cellular responses as part of their evil plan to do as much damage to the cells as possible, like the villains in a *Die Hard* movie.

- **The heat shock and unfolded protein response pathways** (Fig. 5) detect unfolded or misfolded proteins, or other strange protein shenanigans in the cytoplasm (heat shock response) and in the endoplasmic reticulum (unfolded protein response). Many intracellular pathogens trigger these pathways due to production of pathogen proteins inside cells. In some cases, the resulting response helps to combat the pathogen, while in other cases pathogens actually use that response as part of their evil scheme.

- **The apoptosis defense pathway** (Fig. 6) is a last line of defense: if cells become infected with pathogens (or even without pathogens, if the cells start to become cancer cells), cells can blow themselves up and try to take the bad guys with them. Wily pathogens generally block the signaling pathways that would lead to apoptosis or cellular suicide, keeping infected host cells alive long enough for pathogens to replicate inside and then escape from those infected cells.

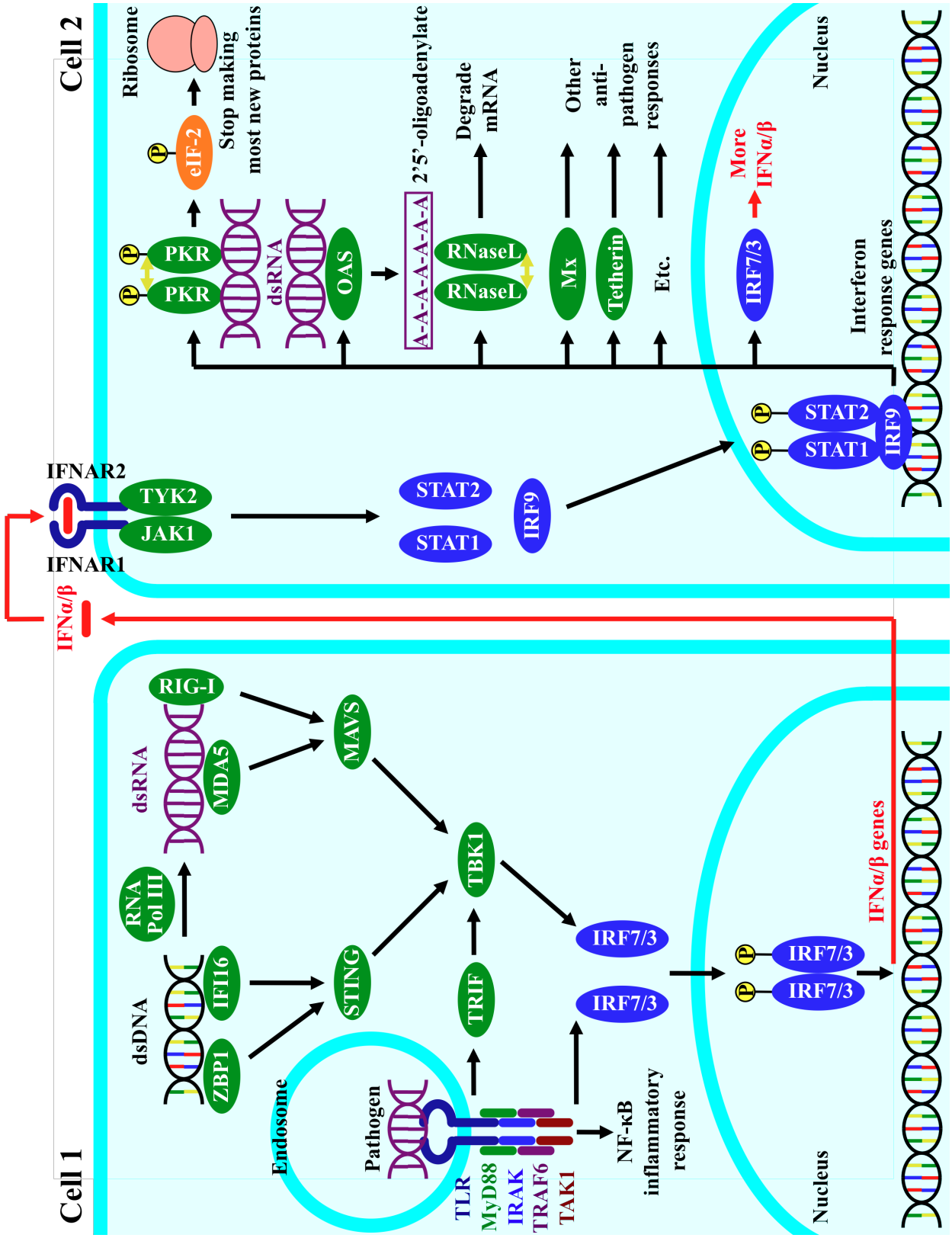


Fig. 3. Interferon defense pathway against microorganisms.

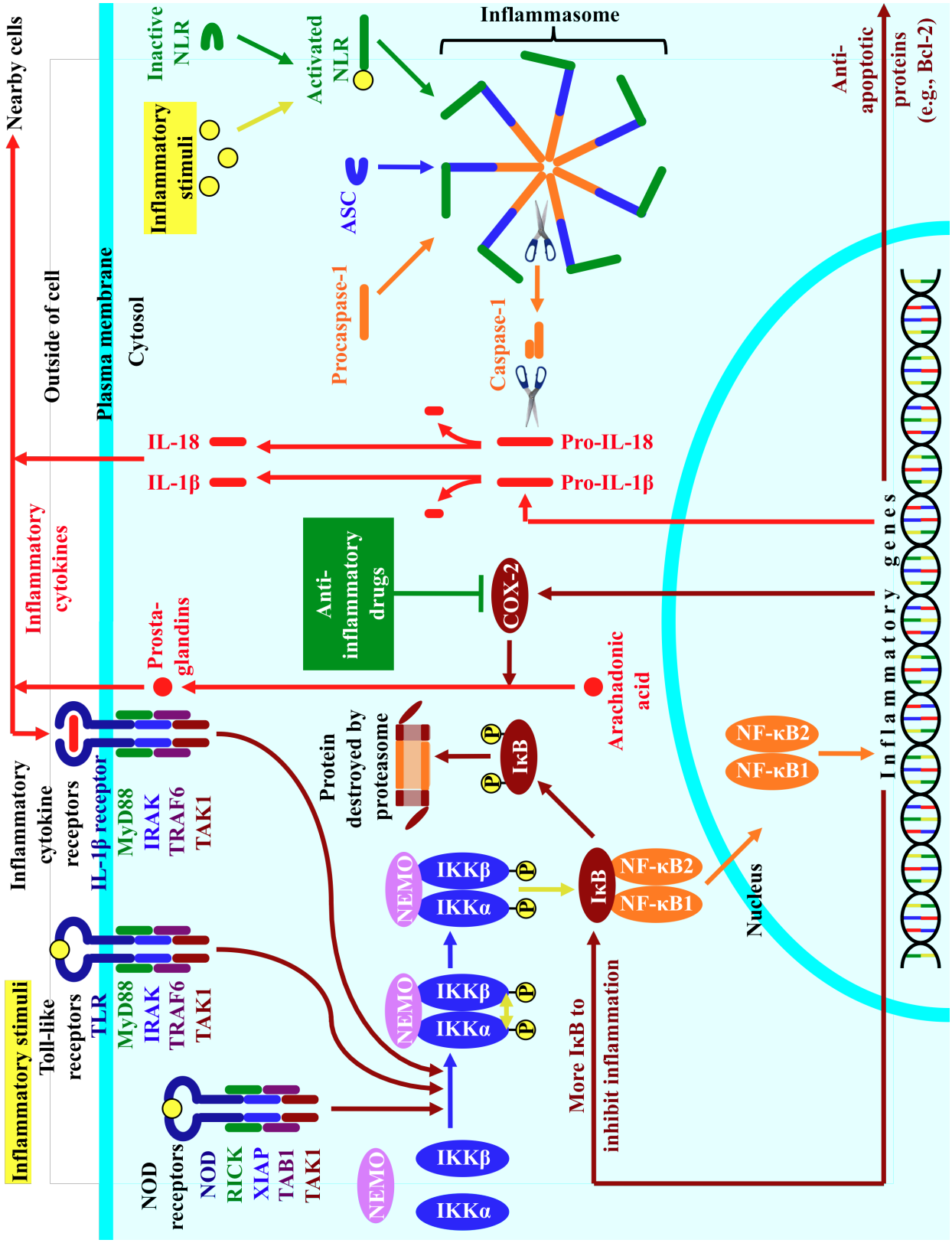


Fig. 4. Inflammatory defense pathway against microorganisms.

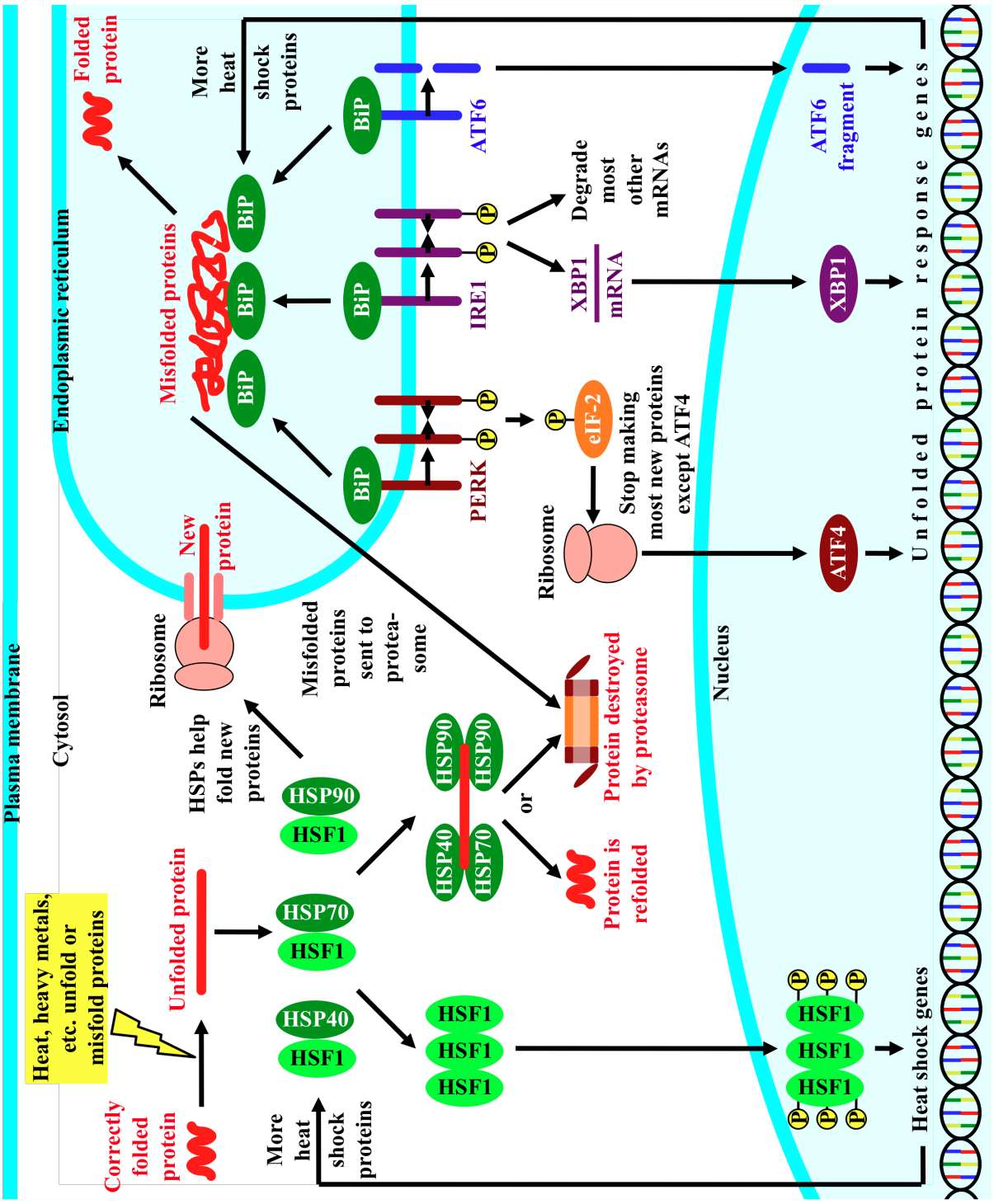


Fig. 5. Heat shock and unfolded protein response pathways affected by some microorganisms.

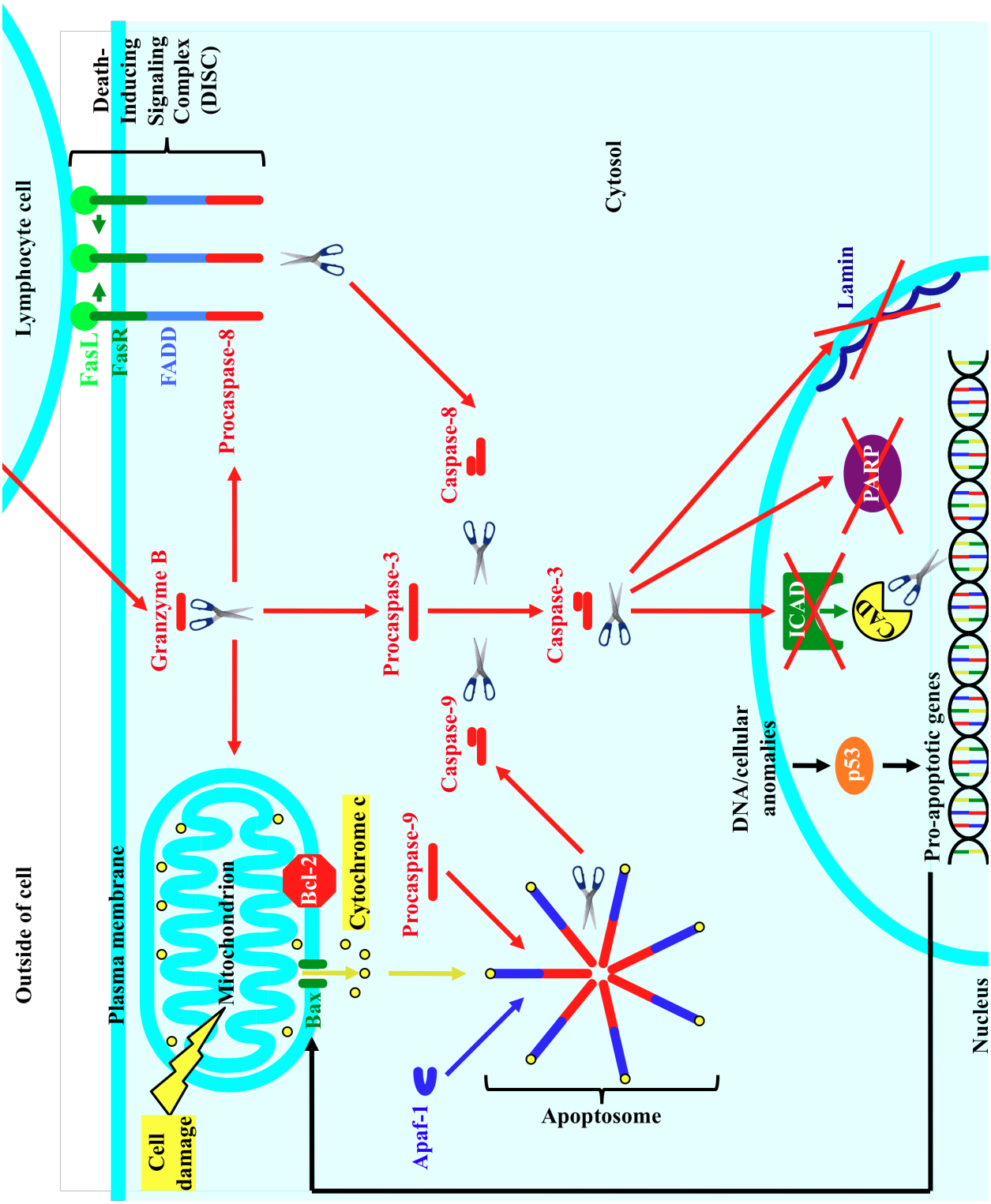


Fig. 6. Apoptosis defense pathway against microorganisms.

Extracellular defenses will be covered in detail in *Immunology*, but some of the major extracellular defenses are shown in Fig. 7 and include:

- **T lymphocytes** peer inside other cells to see if they have been infected by pathogens. If so, the T lymphocytes or T cells kill those cells and hopefully also the pathogens inside them. T cells can even activate the apoptosis pathway to persuade infected cells to kill themselves if the infected cells don't feel guilty enough to do the deed on their own. The most effective pathogens are those that can hide inside cells without being detected until it is too late to stop their evil scheme.

- **B lymphocytes** try to find and clobber pathogens outside our cells. B lymphocytes or B cells make antibodies, proteins that can be tailored to bind to specific pathogens but not anything else. Different B cells have different antibodies on their surface. If the antibodies on the surface of a B cell detect and bind to a specific invading pathogen, that B cell will start replicating and cranking out lots of those same antibodies to attack the pathogens. Sneaky pathogens try to avoid that response by hiding inside our cells or inside other protective enclosures, and/or by frequently changing their outer appearance so a given set of antibodies will no longer recognize them.

- **Other immune system cells** such as natural killer (NK) cells and macrophages destroy cells that fail to show proper identification, since they assume that those may be pathogen cells, host cells that are infected but trying to hide it, host cells that are becoming cancer cells, or other cells that are up to no good.

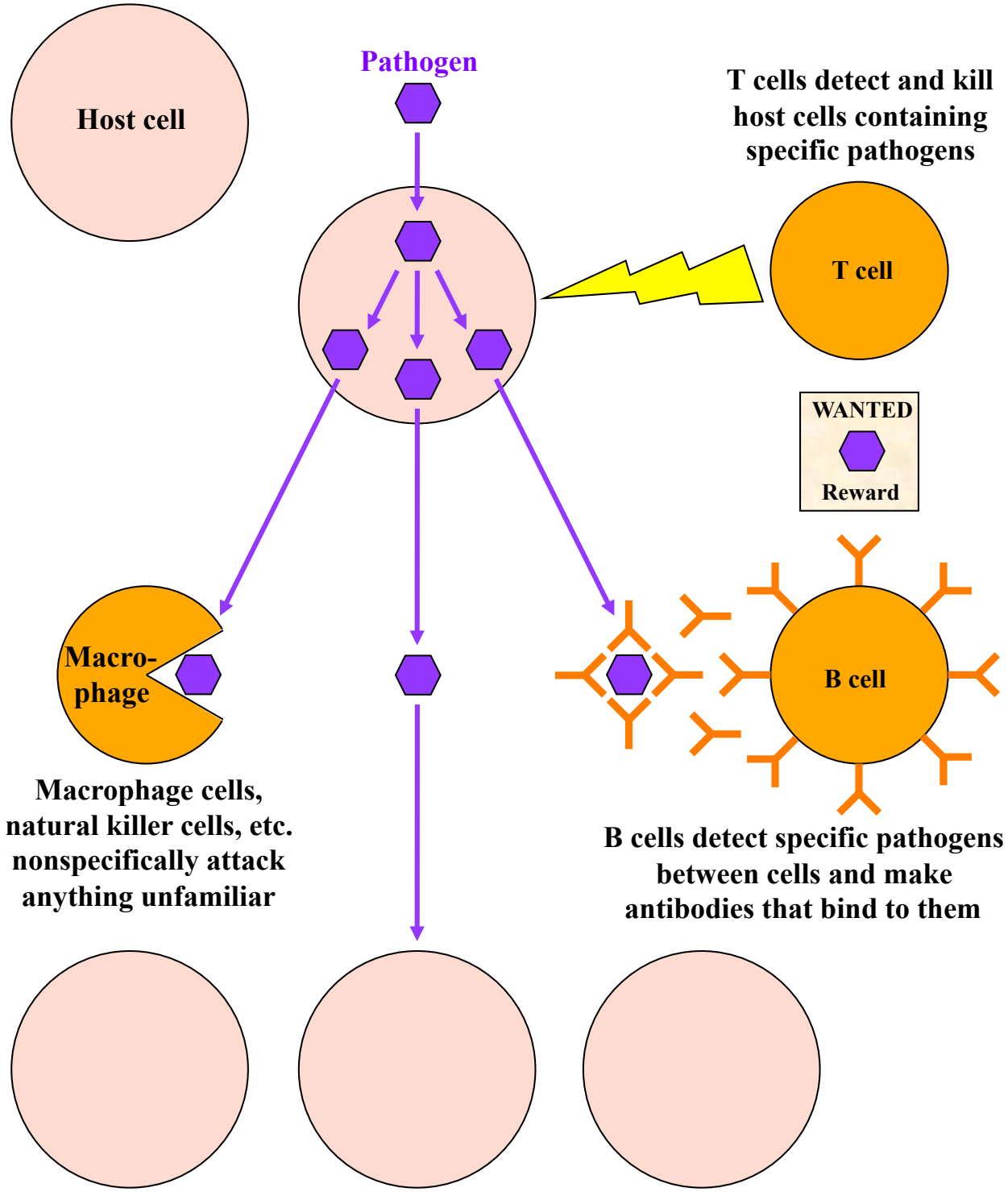


Fig. 7. Extracellular or immune defense pathways against microorganisms.

1.3 Experimental Methods

The particular methods used in microbiology experiments depend on the microorganisms being studied, but some common techniques are shown in Figs. 8-9:

(a) Solid and liquid culture media let us grow many kinds of microorganisms. To be more scientific than all of that stuff growing in the back of your refrigerator, we can use solid or liquid nutrient media with ingredients that are great for growing specific types of microorganisms but not anything else. That way if something grows, you have a fairly good idea what it is.

(b) Antibiotic or other drug sensitivity assays test the ability of microorganisms to grow in the presence of different concentrations of various antibiotics (which kill most bacteria) or other drugs (that might kill types of microorganisms). This information helps to identify what critters you are growing, and of course it is also helpful if you need to know how to wipe it out in an infected patient, assuming the patient lasts longer than the assay.

(c) Staining for microscopy makes it a heck of a lot easier to see very small, very transparent beasties under the microscope. For bacteria, there are a number of different stains or colored dyes that are taken up by certain types of bacteria but not others, which helps to tell them apart if you can't immediately put a name to each face.

(d) Enzyme assays involve adding different chemical molecules to test if the microorganism has enzymes (such as catalyses, coagulates, DNases, oxidases, ureases, or pizza-ases) that act on those molecules, in order to identify the microorganism or to study its properties.

(e) Polymerase chain reaction (PCR) is a widely used method of copying DNA, and it can identify or quantitate what pathogen DNA (or RNA) is present. Short single strands of DNA called primers are designed with nucleotide sequences that will specifically bind to each end of a known pathogen gene, yet not bind to anything else. If that pathogen gene is not present in a sample, those primers will have nothing to bind to, and nothing will happen. However, if a pathogen gene is present in a sample, the primers will bind to each end of the gene, an added polymerase enzyme will fill in between the primers to copy the gene, and then the primers and polymerase enzyme can make copies of the gene copies, and copies of the copies of the copies. That chain reaction sets off alarm bells in your DNA copying gizmo.

(f) Immunoassays use antibodies (proteins made by hard-working B lymphocytes as discussed in Section 1.2) that bind to specific pathogens but nothing else. Each antibody is Y-shaped, with an identical pathogen binding site at the top of each prong of the Y. If the corresponding pathogen is not present in a sample, nothing happens, but if it is, antibodies and pathogens bind to each other and create a detectable little clump of goo.

(g) Infection of mammalian cells is a very common experimental technique. To identify a pathogen, or to study how a pathogen works, you can add a smidgen of the potential pathogen to a Petri dish of cells that the pathogen should be able to infect, then peer through the microscope and see if a panic ensues.

(h) Infection of animal models is also a common technique. Detailed studies of how a pathogen does its dastardly deeds, and how drugs might be able to treat that, require testing in live animals, where the pathogens and any potential drugs can interact with all the various cell types in the animal, not just one cell type in a Petri dish. That is bad for cute little furry animals, but good for countless humans who might be saved by what we can learn this way.

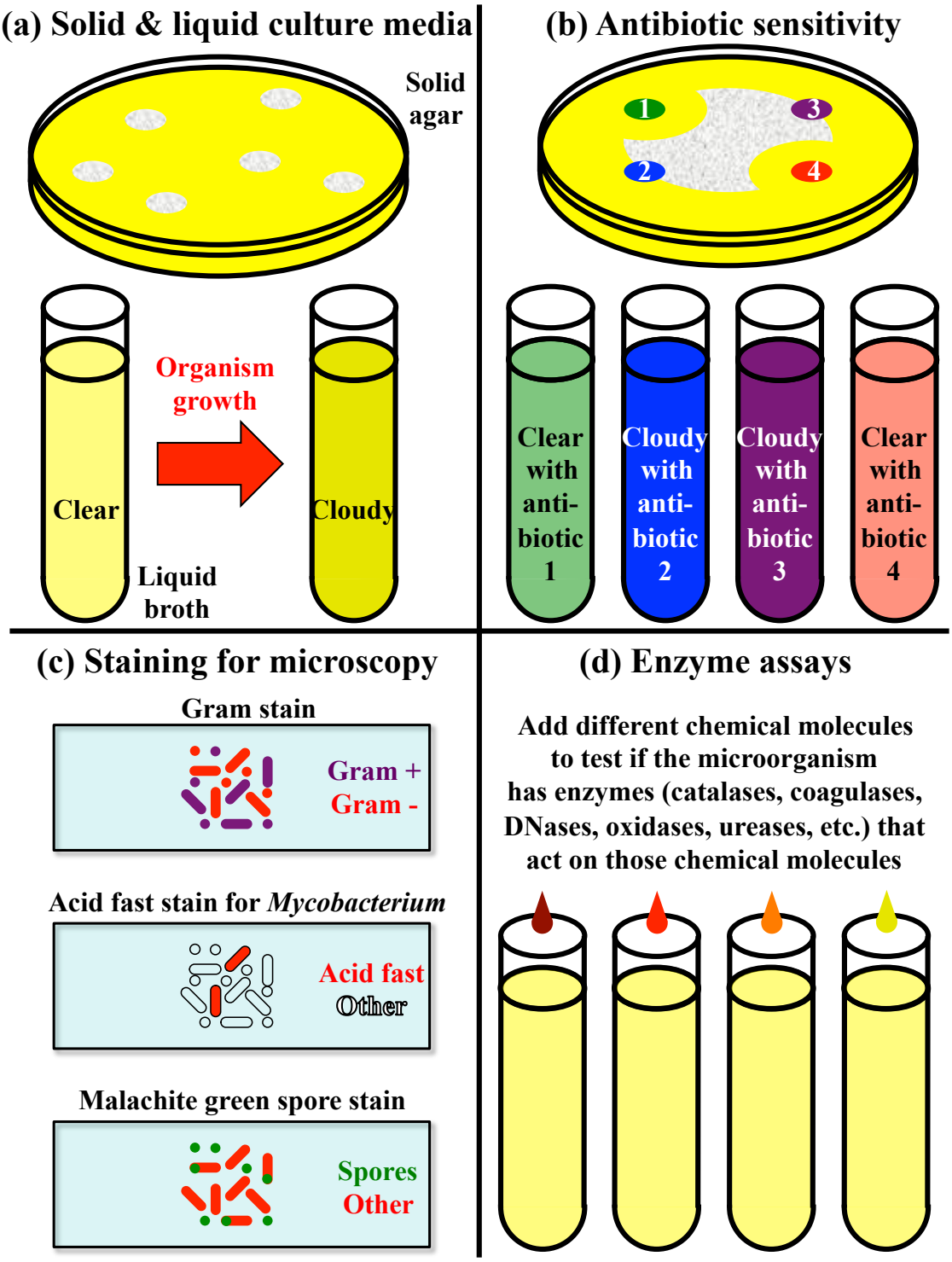


Fig. 8. Some experimental methods used in microbiology, including **(a)** selective solid and liquid culture media, **(b)** antibiotic or other drug sensitivity assays, **(c)** selective staining for microscope observations, and **(d)** biochemical assays to test for the presence of different enzymes.

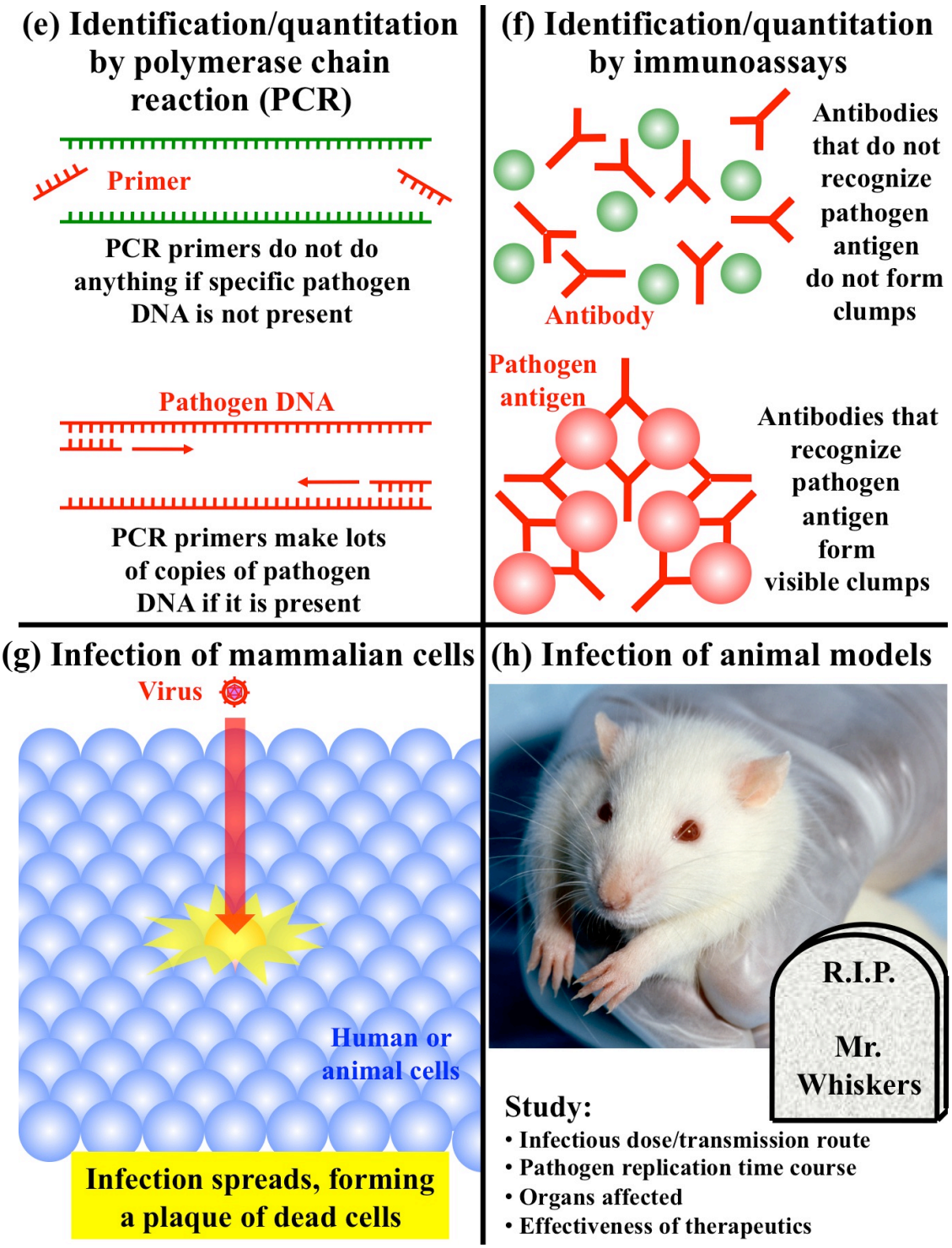


Fig. 9. More experimental methods used in microbiology, including **(e)** identification and quantitation by polymerase chain reaction (PCR), **(f)** identification and quantitation by immunoassays, **(g)** infection of mammalian (human or animal) cells, and **(h)** infection of animal models.

2 Prions

Prions are just rogue proteins and not real live microorganisms, but they can spread from one animal or human host to another and kill their hosts, so we will consider them as part of microbiology. To replicate, prions persuade some of the host's own proteins to change their shape and turn into new prion proteins. The best-known prions will Swiss-cheese brains, causing cows to get mad and cannibals to get wild and crazy.

2.1 Classification and Mechanisms of Prions

The normal, healthy form and the pathogenic form of a prion protein (PrP) have the same amino acid sequence as each other, but they are folded up differently. The left side of Fig. 10(a) shows the normal folded structure of a typical healthy PrP protein, with large comforting green regions of alpha helices (organized spirals of amino acids), small blue arrows for regions of beta sheet (organized amino acids forming a two-dimensional flat structure), and yellow regions with loops or relatively little order (just strings of seemingly random amino acids). The right side of Fig. 10(b) shows the refolded structure of a pathogenic PrP protein. Despite having the exact same amino acid sequence as the healthy version, the pathogenic version has significantly less alpha helical structure (green) and a lot more beta sheet structure (blue arrows).

Figure 10(b) illustrates how pathogenic prions can recruit initially healthy PrP proteins to convert them into more prions. Pathogenic prions, shown in red, tend to accumulate to form large clumps, called fibrils or plaques. Some pathogenic prions can break off from the clumps and bind to healthy prion proteins (green) normally found in the host animal or person. The pathogenic prions refold the initially healthy prion proteins into the pathogenic shape, thus gaining more converts for their cause and multiplying exponentially. If even a small number of pathogenic prions are introduced into a healthy animal or human host (for example, if the new host consumes prion-containing meat from a previous infected host), over time they can convert most of the host's prions into the pathogenic form.

Figure 11(a) shows how prions cause disease (pathogenic effects) in a host. Normal healthy prions (shown in green) are produced in neurons (brain cells) and transported to the surface of the cells, where they perform essential functions that are currently not well understood. Pathogenic prions (red) are not bound to cell surfaces, so they are free to travel between cells and between hosts to spread the infection. As pathogenic prions recruit and convert initially healthy prions from the cell surface, the fibrils or plaques of pathogenic prions grow in the space between cells. Some pathogenic prions are internalized from the cell surface to inside the neuron, where they directly or indirectly cause cell death via mechanisms that are still not well understood. By killing some neurons and producing fibrils or plaques between the surviving neurons, prions cause spongiform encephalopathy in the brain, like the Swiss-cheese-like holes in the prion-infected cow brain shown in Fig. 11(b).

Because of their unique folded structure, pathogenic prions are tough little buggers, able to survive everything from cooking of meat to lying on the ground exposed to the elements for years. Do you really know where your burger has been?

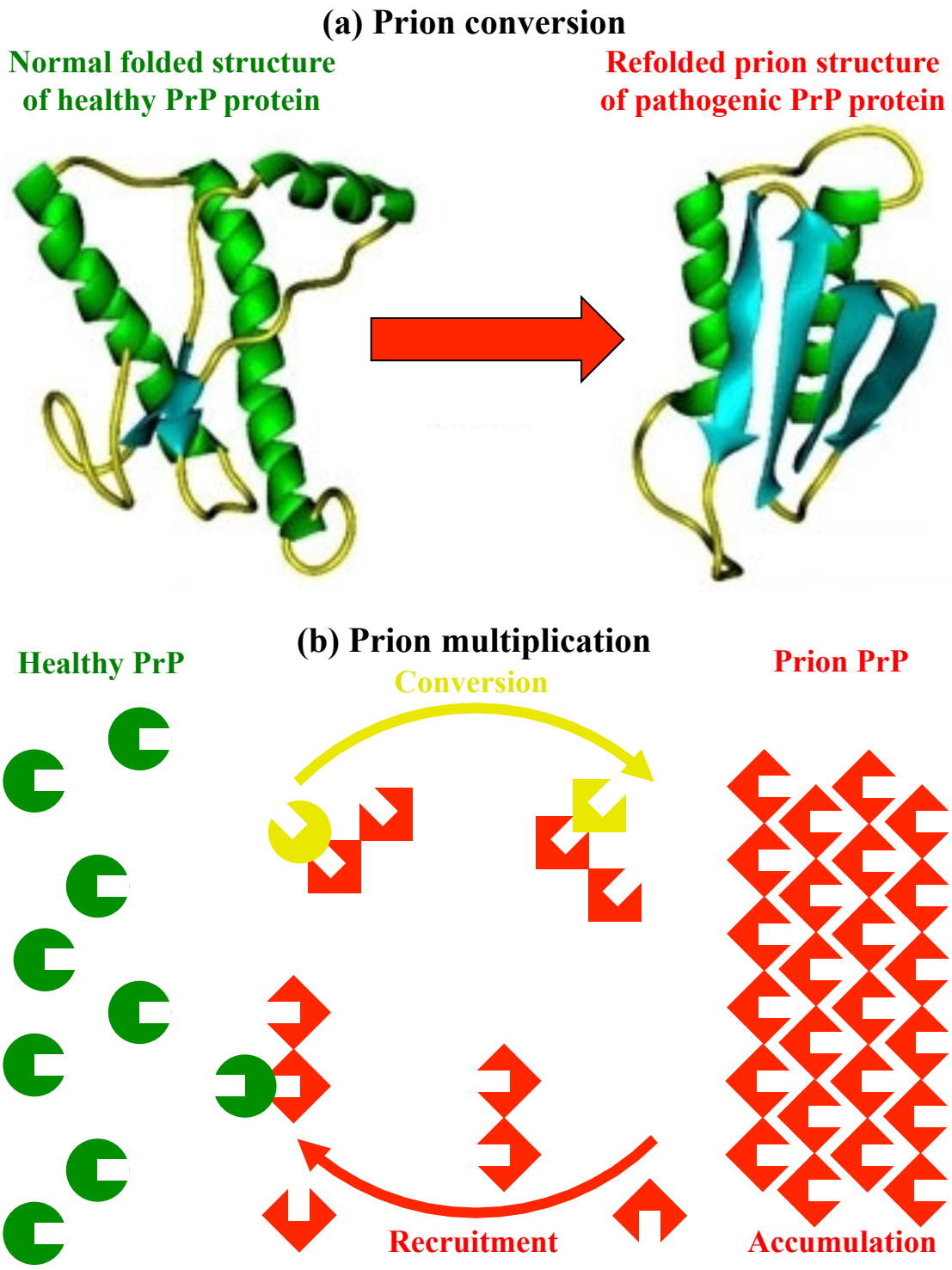


Fig. 10. The mechanism of pathogenic prion formation, showing (a) how a healthy PrP protein can be refolded to be a pathogenic PrP protein, and **(b)** how pathogenic prions can recruit initially healthy PrP proteins to convert them into more prions.

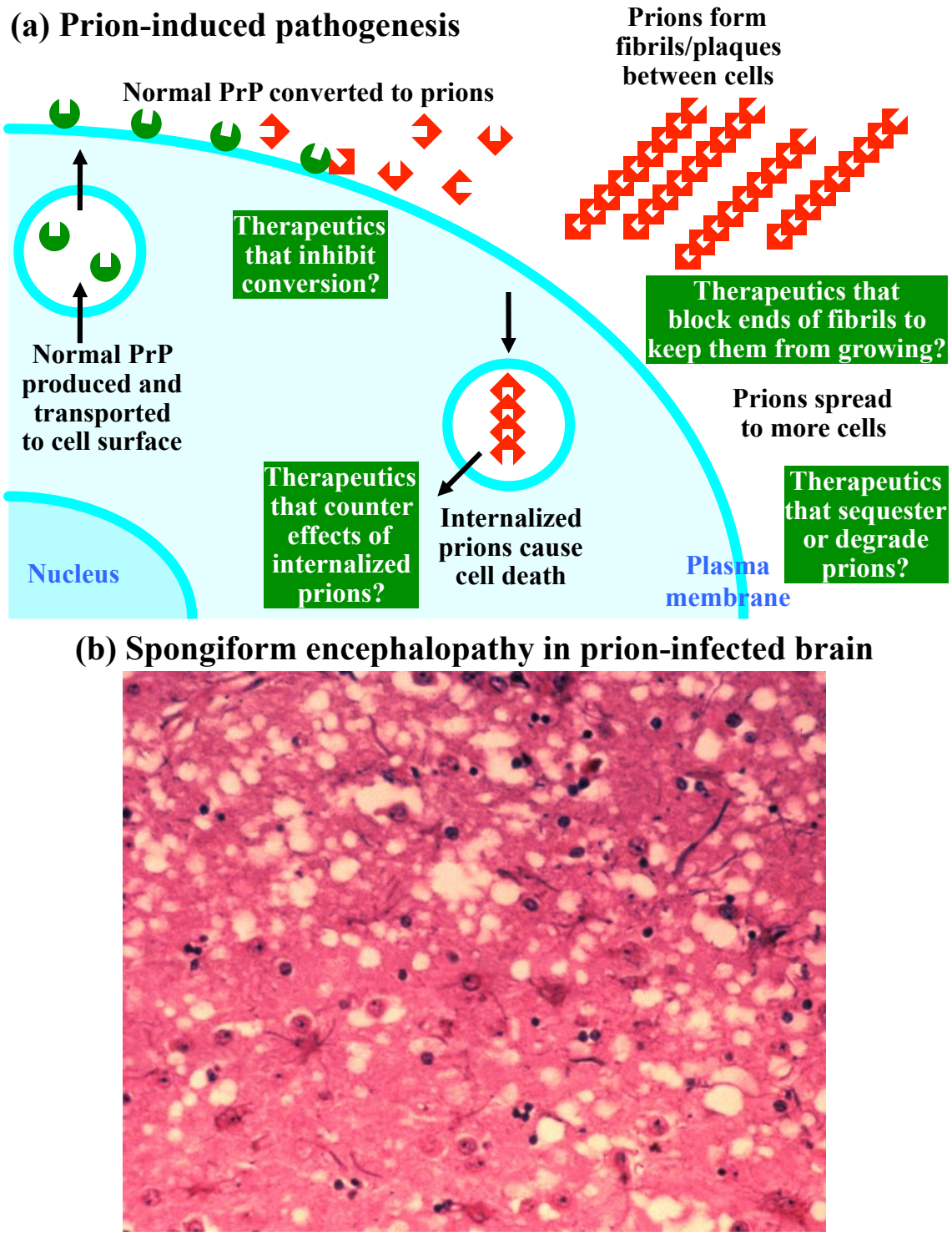


Fig. 11. Pathogenic effects of prions, showing (a) how prions cause damage to cells and **(b)** spongiform encephalopathy or "Swiss cheesing" in a prion-infected cow brain.

Some diseases that are caused by or at least somehow involve prion-like proteins include:

- **Bovine spongiform encephalopathy (BSE)** or mad cow disease, caused by bovine PrP protein.
- **Chronic wasting disease** in deer and elk, caused by deer/elk PrP protein.
- **Scrapie** in sheep and goats, caused by sheep PrP protein.
- **Creutzfeldt-Jakob disease (CJD), Kuru, BSE**, or other infectious prion diseases in humans, caused by human PrP (which may be triggered by bovine, deer, or elk PrP protein, but apparently not sheep PrP protein, which is different enough in its amino acid sequence).
- **Fatal familial insomnia, familial CJD**, and other similar genetic disorders, caused by inherited mutations in human PrP proteins that predispose them to convert to the pathogenic form.
- **Alzheimer's disease**, a neurodegenerative disease that affects memory, whose initial cause is not well understood but whose progression seems to involve prion-like aggregates of beta amyloid and tau proteins.
- **Parkinson's disease**, a neurodegenerative disease that causes paralysis, also not well understood but somehow involving prion-like aggregates of alpha-synuclein protein.
- **Amyotrophic lateral sclerosis (ALS)**, another neurodegenerative disease that causes paralysis, which somehow involves aggregates of TAR DNA-binding protein 43 or TDP-43.
- **Huntington's disease**, an inherited neurodegenerative disease that affects both memory and motor control and that involves aggregates of mutant Huntingtin protein.
- **Chronic traumatic encephalopathy (CTE)**, a neurodegenerative disease that affects memory and mood, is triggered by significant head trauma (as in boxers, American football players, or soldiers) and whose progression involves prion-like aggregates of beta amyloid, tau, and/or TDP-83 proteins.

2.2 Anti-Prion Therapeutics

Although there are no existing therapeutics for prion diseases, there are several potential approaches that may ultimately yield effective treatments. Therapeutics might act at various points in prion pathogenesis, as illustrated in Fig. 11(a):

- Therapeutics that inhibit conversion, for example by reducing the amount of normal prion protein (if that can be done without causing deleterious effects) or by effectively making it more resistant to conversion to the pathogenic prion structure.
- Therapeutics that block the ends of fibrils to keep them from growing, for example antibodies or small-molecule drugs that specifically bind to the ends of pathogenic prion fibrils.

- Therapeutics that sequester or degrade pathogenic prions, for example antibodies or small-molecule drugs that specifically glom onto free pathogenic prions and/or target them for destruction by cellular mechanisms of protein degradation.

- Therapeutics that counter the effects of internalized prions, for example by preventing prion-induced cell death or by acting on the heat shock and unfolded protein response pathways. These pathways (Fig. 5) detect unfolded or misfolded proteins in the cytoplasm and endoplasmic reticulum, and attempt to properly refold or else destroy as many misfolded proteins as possible. Presumably they act to slow the effects of prion and prion-like diseases but get overwhelmed by the sheer number of misfolded pathogenic proteins. Future therapeutics may be able to aid heat shock/unfolded protein response pathways in combatting misfolded pathogenic proteins, or alternatively to prevent these pathways from reacting incorrectly to the misfolded pathogenic proteins.

2.3 Useful Applications of Prions

Some prion-like natural processes are actually good for you:

- Inflammasomes that detect and respond to inflammatory signals in cells (Fig. 4) are formed by NOD-like receptors (NLRs) and Apoptosis Speck-like CARD (ASC) adaptor proteins that change shape upon detecting inflammatory signals, causing more NLR and ASC proteins to change shape, until they all form an aggregated wheel-like structure and activate procaspase-1.

- Likewise, apoptosomes that detect and respond to cell suicide signals in cells (Fig. 6), are formed by Apaf-1 proteins that change shape upon detecting cytochrome c released by damaged mitochondria, causing more Apaf-1 proteins to change shape, until they all form an aggregated wheel-like structure and activate procaspase-9.

- Some receptors such as toll-like receptors (TLRs) activate a series of proteins (such as MyD88, IRAK, and TRAF6) that create a polymerized scaffold for receptor signaling, in a process analogous to prion aggregation.

- Some natural cellular proteins act as kinases, enzymes that covalently attach chemical phosphate groups to certain other proteins. In cell signaling pathways that control cell division, there are cascades or chain reactions of one kinase phosphorylating and activating another kinase, which activates another, and so on to infinity and beyond. While that involves chemical modification of the proteins and real prions technically do not, this process does involve one protein changing its folded state (controlled by the presence or absence of the negatively charged phosphate group) and ultimately causing all the other proteins to do so too.

- Similarly, some natural proteins act as proteases, enzymes that cut certain other proteins at specific locations, and in some biochemical pathways there are cascades or chain reactions of proteases, with one protease cutting and thereby activating more proteases. Again, this is a chemical modification (cutting) and not purely a simple refolding of the proteins as in true prions, but the proteins in a proteolytic cascade do catalyze each other's transitions from one state to the other. Examples of important proteolytic cascades include caspases in the apoptosis pathway (Fig. 6), complement proteins in the immune system, and proteins in the blood clotting cascade.

3 Viruses

Viruses are rogue genes that invade host cells, command cells to make more copies of the viral genes, and usually (but not always) kill the host cells on the way out, before spreading to infect more host cells. They include everything from the common cold to Ebola.

3.1 Classification and Mechanisms of Viruses

Figure 12 shows the innards of your average garden-variety viruses. Viruses can range in size from smaller than 20 nm (for parvoviruses) to larger than 350 nm (for smallpox), with ∼50 nm as a typical average size. Viruses generally include the following components:

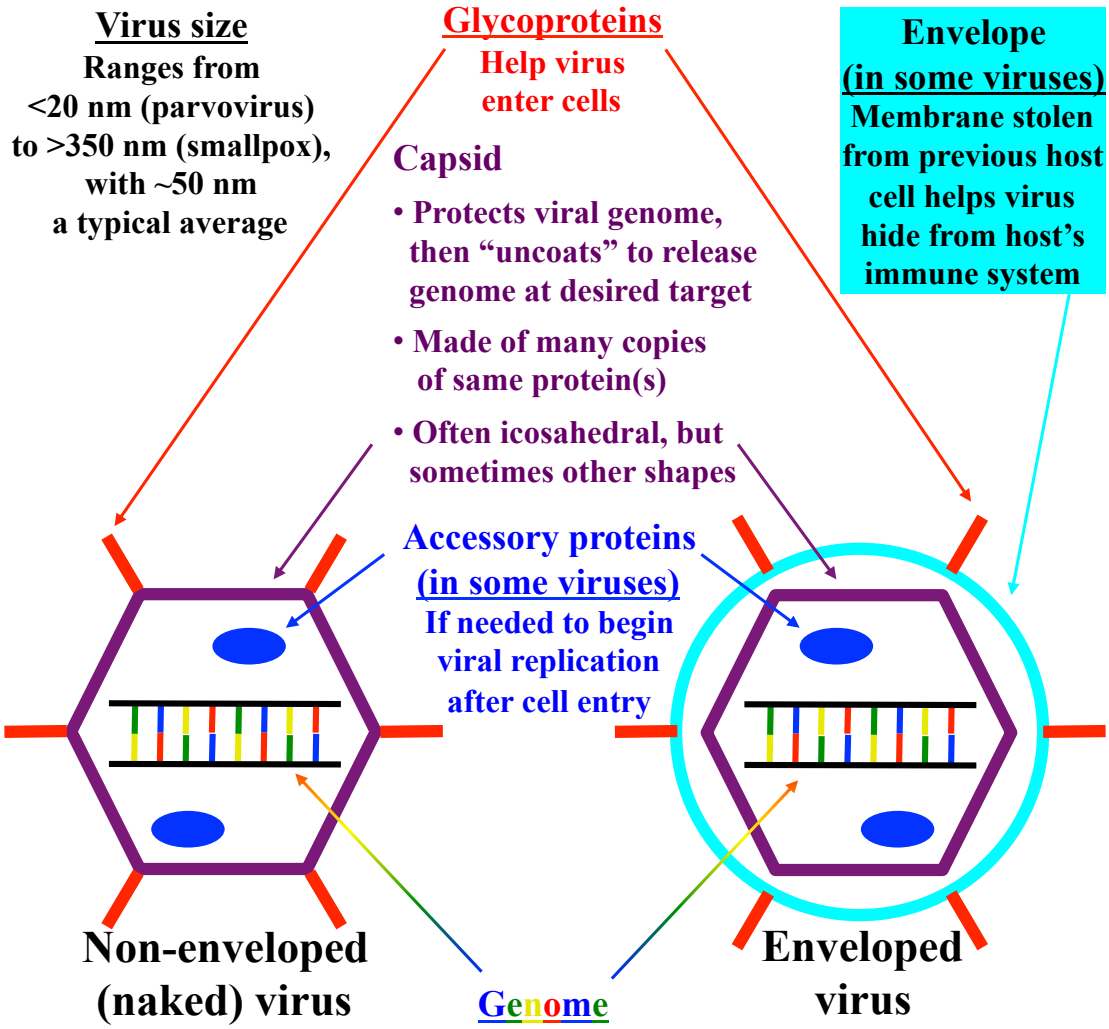
- The **viral genome** or complete set of viral genes is the most important part of the virus, just as hot air is the most important part of the politician. The majority of viruses use RNA to encode their genome, although more pretentious viruses use DNA just like we do. The genome may be double-stranded (ds) or single-stranded (ss), depending on the specific type of virus. It is usually all in one linear or circular segment, but a few viruses have genomes consisting of multiple independent segments. The viral genome usually encodes only a few genes, such as those for structural viral capsid proteins and glycoproteins, accessory or other proteins for replication, and countermeasures against the host's defenses. Viral genomes range in size from less than 1700 nucleotides or bases for hepatitis D to over 180,000 bases for smallpox, but ∼10,000 bases is a good average for most viruses.

- Some viruses package **accessory viral proteins** along with their genome, if they are needed to begin viral replication after the virus enters a host cell. Otherwise viruses make use of natural cellular proteins or use their viral genomes to encode replication proteins that get made by the host cell.

- The viral genome (and accessory proteins, if they exist) are surrounded by the **viral capsid**, a protein coat that protects the viral genome when it is in transit from one cell to another, or from one host organism to another. Sometimes the viral nucleic acid genome and protein capsid are collectively called the nucleocapsid. The viral genome uncoats or removes its protective capsid after it has entered a new host cell and arrived at its desired location. Capsids are made of many copies of the same protein or proteins, all self-organized into a cute shape. Capsids are often icosahedral (viruses are big fans of Buckminster Fuller) but can be other shapes depending on the specific type of virus.

- To try to hide from the host's immune system, some viruses wrap their nucleocapsid in lipid membrane stolen from the virus's previous host cell, like a wolf in sheep's clothing. This membrane is called the viral envelope, and viruses that use it are called enveloped viruses. Viruses that get by just fine without a lipid membrane are called non-enveloped viruses, or naked viruses if you really want to make them feel self-conscious.

- Most viruses have glycoproteins (proteins with attached sugars) on their outside, either protruding from the nucleocapsid for non-enveloped viruses or protruding from the lipid membrane for enveloped viruses. The glycoproteins help the viruses attach to and enter host cells.



- RNA (most viruses) or DNA (other viruses)
- Double-stranded (ds) or single-stranded (ss)
- Usually all in one segment (but influenza has 8 segments); may be linear or circular
- Usually encodes only a few genes
  - Capsid proteins and glycoproteins
  - Accessory or other proteins for replication
  - Countermeasures against host defenses
- Ranges from <1700 bases (hepatitis D) to >180,000 bases (smallpox), with ~10,000 bases a typical average

Fig. 12. General structures of typical non-enveloped and enveloped viruses.

For viruses to convert their genes into proteins inside host cells, they must make use of the natural cellular pathways for transcription and translation from genes to proteins, as shown in Fig. 13:

- DNA is a natural biological linear polymer containing a sequence of four possible subunits (bases or nucleotides). DNA is a relatively long-lived and precise molecule for encoding large amounts of information, and thus it is used in the cell nucleus (or nucleoid in prokaryotes) to store, replicate, and control the genes in all organisms, except some viruses. DNA regions may be classified as exons or introns (which both end up being copied to RNA), promoters (which control how many RNA copies to make), and intermediate regions that serve as spacers or perform other functions. DNA is **replicated** when a cell divides to become two cells. Viruses with DNA genomes must replicate their viral DNA, and they usually use natural cellular enzymes in the nucleus to do that.

- RNA is chemically very similar to DNA and also has a sequence of four possible subunits (bases or nucleotides), but it is a less long-lived and somewhat more error-prone biomolecule for encoding information. However, inside the nucleus, cells **transcribe** their individual genes from DNA to RNA to make temporary working copies of genes. A majority of viruses only store their genes as RNA, so they have to have their own way of replicating their RNA genomes, since RNA replication is not normally done in cells.

- Still inside the nucleus, after a cell makes messenger RNA (mRNA) copies of individual genes, those mRNAs undergo **post-transcriptional modification**, including splicing to remove their introns yet leave their exons, and addition of a 5' cap on one end and 3' poly-adenosine tail on the other end to stabilize them. Then the mRNAs are shipped out of the nucleus. Viruses must use and/or mimic these natural cellular RNA processing methods.

- Ribosomes outside the nucleus **translate** the RNA nucleotide sequences of individual genes to create proteins of the correct corresponding amino acid sequences. Proteins are polymers containing sequences of at least 20 possible subunits (amino acids or residues), and they function as specialized molecular machines to perform most of the essential tasks in and around cells. Viruses rely on cellular ribosomes to produce all of their viral proteins.

- To prepare for their new jobs, some proteins require **post-translational modification** in the endoplasmic reticulum and Golgi apparatus. These modifications include glycosylation or adding glycan (sugar) groups, creating disulfide bridges, phosphorylation or adding phosphate groups, and any other modifications required to help the proteins fold up into their final functional form. Some viral proteins are processed through these pathways, and in fact some viruses like to set up residence inside the endoplasmic reticulum and replicate themselves there.

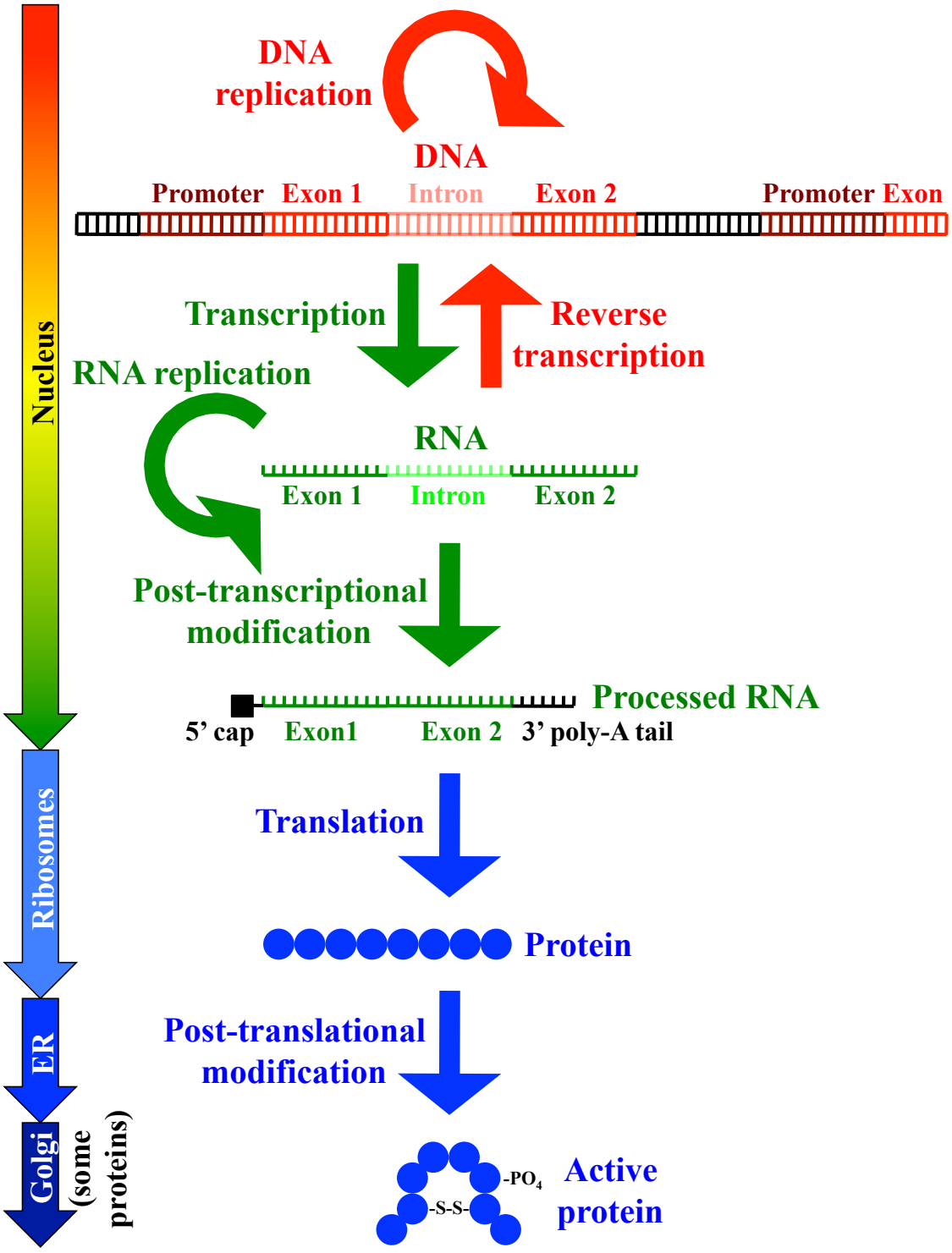


Fig. 13. Viruses make use of the natural cellular pathways for transcription and translation from genes to proteins.

Viruses have families, because otherwise no one else would love them. Virus families are classified into groups based on what type of genome they have (Figs. 14 and 15). This is called the Baltimore classification system, but it works well in other cities too:

Group I: Double-stranded DNA viruses have dsDNA genomes just like host cells. As shown in Fig. 14, they usually use a host DNA polymerase to replicate their DNA (although some are picky and use their own viral DNA polymerase), and they use a host RNA polymerase to translate their genes into mRNA just like ordinary folks. Some major dsDNA viruses are shown in Fig. 15:

- **Bacteriophages**, or viruses that infect bacteria, mostly fall in the dsDNA group, although some have RNA genomes. They are non-enveloped and most have the very clever structure shown in Fig. 16. A bacteriophage can cause a **lysogenic** infection, in which the phage genome is incorporated into the bacterial chromosome and remains dormant but gets replicated with the rest of the bacterial chromosome each time the bacterium divides. Alternatively, if the phage is in a bad mood, it can decide to cause a **lytic** infection, making lots of bouncing baby phages that burst out of the host bacterial cell and kill the cell.
- **Papillomaviruses** and **polyomaviruses** are non-enveloped icosahedral viruses that stimulate cell division in host cells that they infect, so that they can use the elevated levels of DNA polymerases in those cells to replicate their own viral genomes. As a result, they can cause anything from warts to cancer. Like most DNA viruses, they replicate in the host cell's nucleus where the host DNA polymerases are, as illustrated in Fig. 17.
- **Adenoviruses** are non-enveloped icosahedral viruses that include several dozen strains that mostly cause respiratory infections, including infections of the adenoids (hence the name).
- **Herpesviruses** are enveloped icosahedral viruses that replicate as in Fig. 17. Similar to what bacteriophages do in bacteria, herpesviruses can cause latent infections (permanently hide in some infected cells with their circular genome acting almost as an extra chromosome in the nucleus) or lytic infections (replicating in and then killing infected cells). Since it is very difficult for the immune system to find and eradicate all of the latently infected cells, herpesvirus infections are usually lifelong; they can be dormant for years and reemerge at any time. The family of herpesviruses includes herpes simplex virus 1 (HSV-1, which mostly causes cold sores), herpes simplex virus 2 (HSV-2, which causes sexually transmitted sores in the nether regions), varicella zoster virus (which generally causes chickenpox in younger people and reemerges in older infected people as shingles), Epstein-Barr virus (EBV, which causes mononucleosis), cytomegalovirus (CMV), and Kaposi's sarcoma herpesvirus (KSHV).
- **Poxviruses** are enveloped brick-shaped viruses that include the highly infectious and frequently deadly smallpox, which infected humans and is now eradicated except for small stocks in labs, and similar viruses that infect other species (monkeypox, cowpox, etc.) The smallpox vaccine or vaccinia was derived from cowpox, which was similar enough to teach the immune system what to look for, but different enough not to cause significant harm in humans. Unlike most other DNA viruses, poxviruses replicate in the cytoplasm and not the nucleus of host cells; poxviruses are so large and have so many genes that they pack and bring almost everything they need, like evil boy scouts.
- **Baculoviruses** are enveloped rod-shaped viruses that infect insects, which might make them useful as natural insecticides as long as your name is not Gregor Samsa. Currently they are mainly of interest because baculoviruses can be engineered with new genes, then added to cultures of insect cells, where they produce very large amounts of the proteins encoded by those new genes.

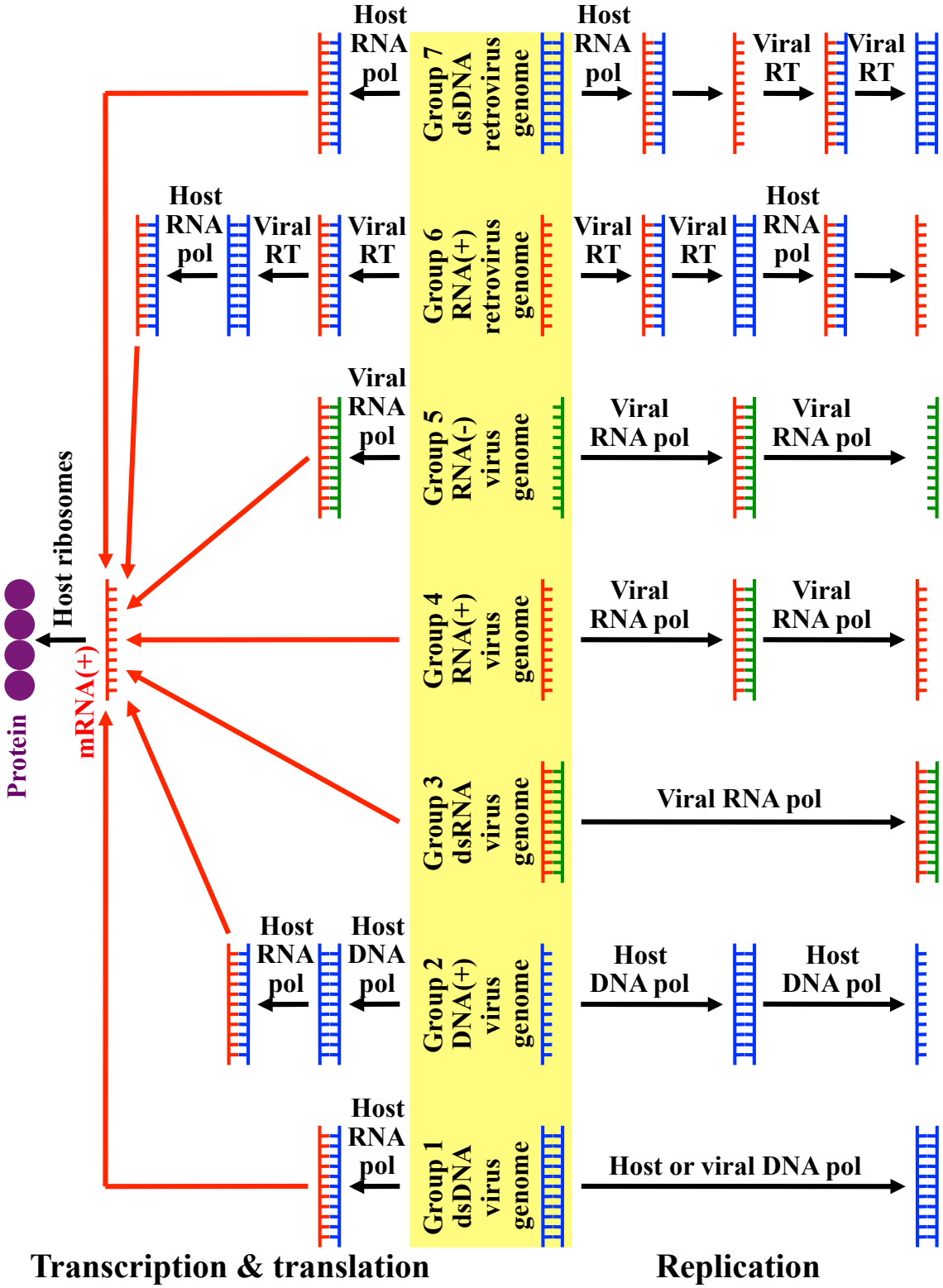


Fig. 14. All known viruses may be classified into seven groups, based on the structure of their genomes. Different groups of viruses use different strategies to replicate their genomes and to produce mRNA encoding viral proteins.

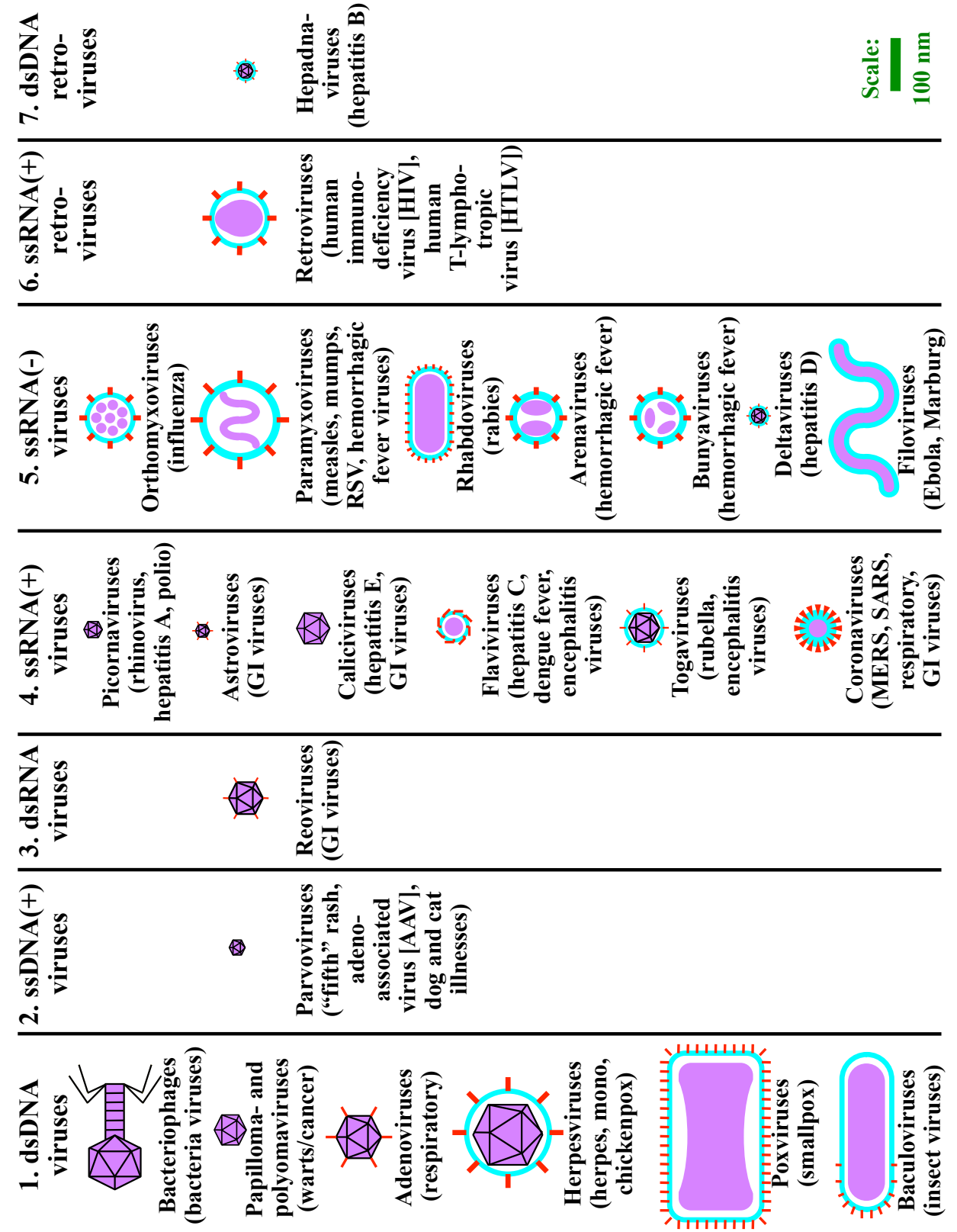


Fig. 15. Virus families within each group that are important for humans (plus bacteria-specific bacteriophages and insect-specific baculoviruses that are useful in molecular biology).

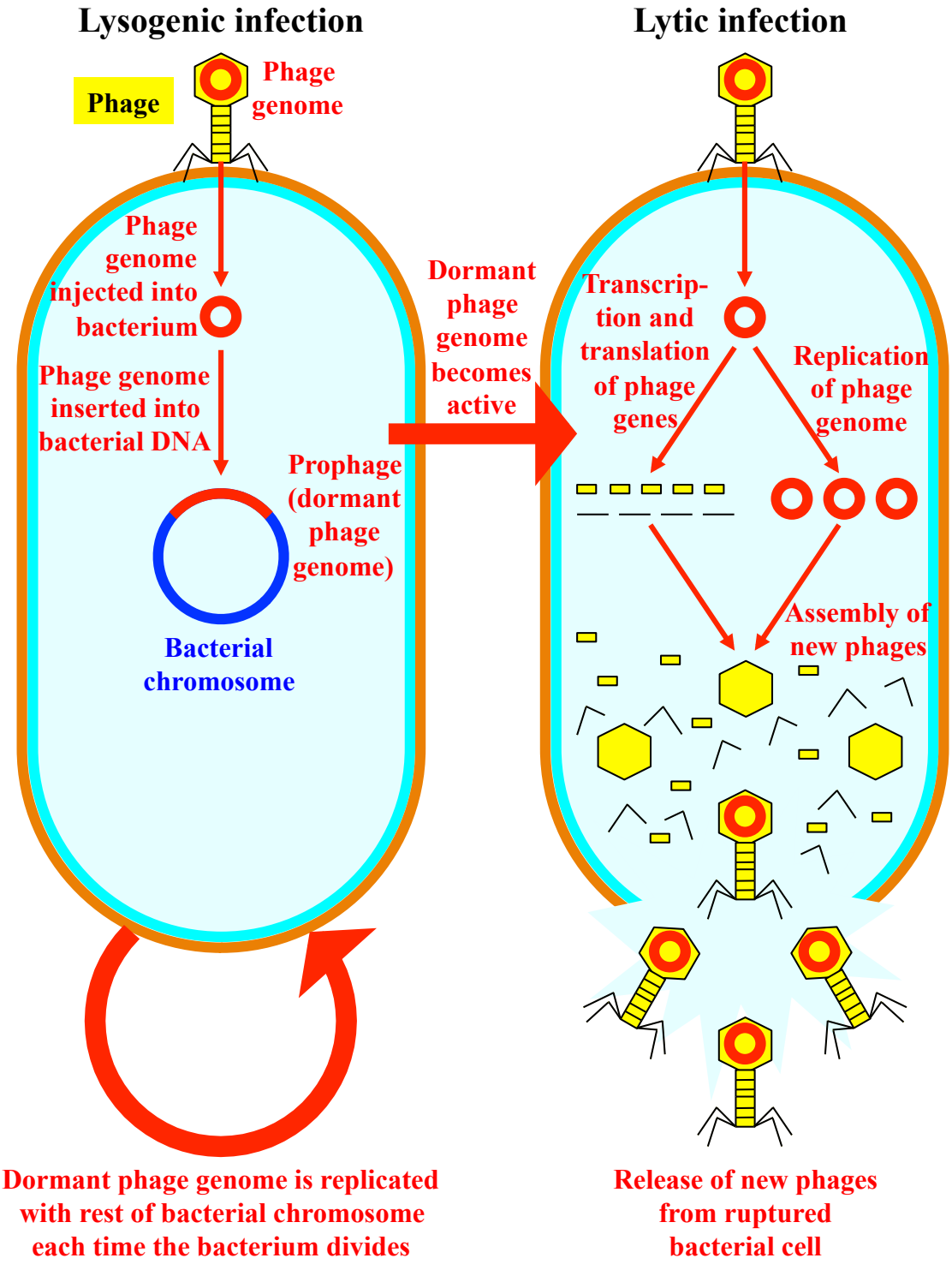


Fig. 16. Replication of a typical bacteriophage. A bacteriophage can cause a **lysogenic** infection, in which the phage genome is incorporated into the bacterial chromosome and remains dormant but gets replicated with the rest of the bacterial chromosome each time the bacterium divides. Alternatively, if the bacteriophage has watched too many *Alien* movies, it can decide to cause a **lytic** infection, making lots of new phages that burst out of the host bacterial cell and kill the cell.

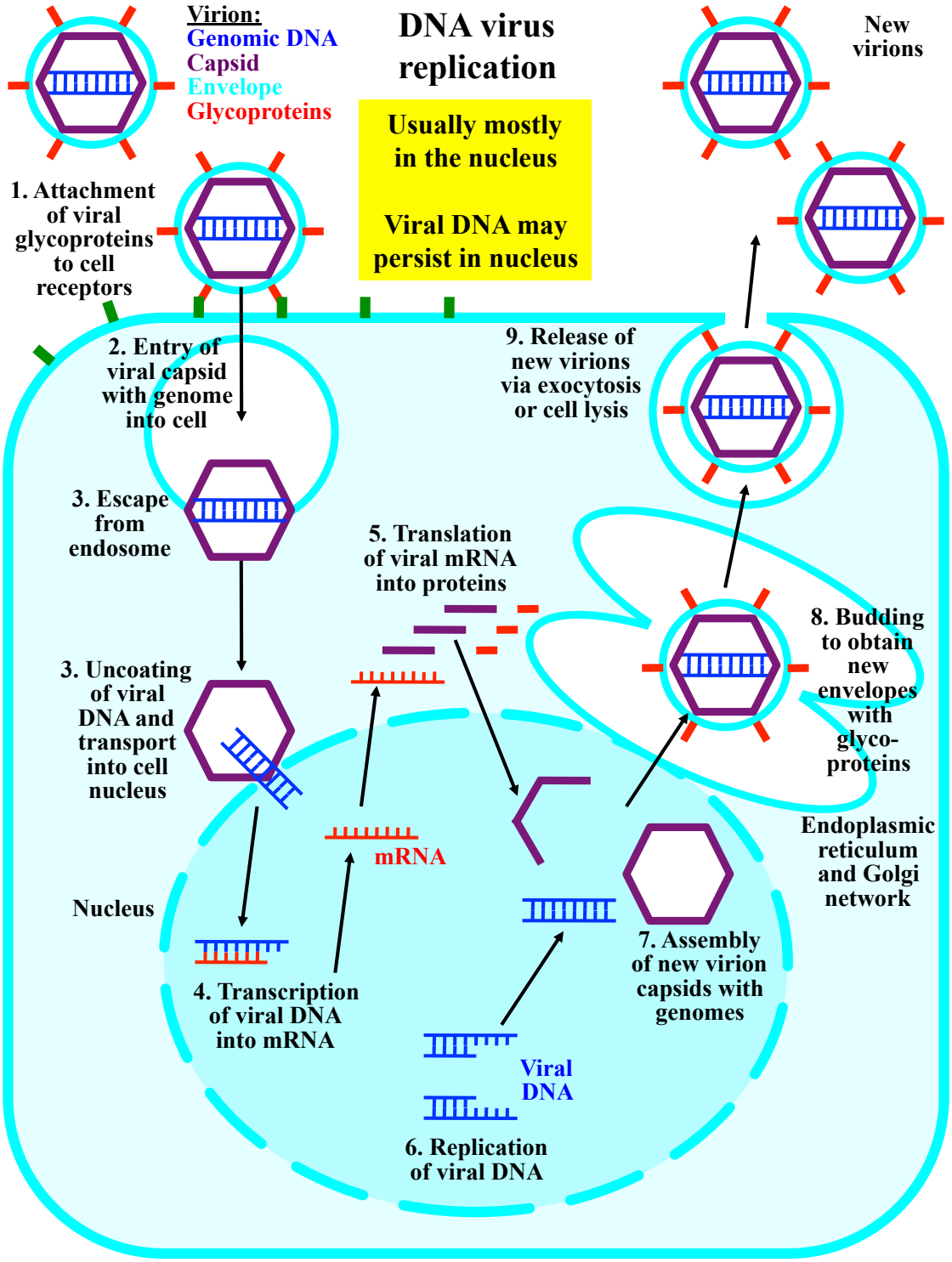


Fig. 17. Replication of a typical DNA virus. Almost all DNA viruses replicate inside the nucleus, since they do need access to the cell's enzymes for replicating DNA and transcribing DNA to RNA. (Poxviruses are a major exception–they come better prepared than boy scouts and set up shop in the cytoplasm with everything they need.)

Group II: Single-stranded DNA viruses only have one strand of DNA, and it is the positive (+) sense strand, the same strand that needs to be mRNA to produce viral proteins. Before +ssDNA viruses do anything else, they use a host DNA polymerase to convert themselves into more respectable looking dsDNA, as illustrated in Fig. 14. For replication, they use that newly created negative (-) sense DNA strand and the host DNA polymerase to make new copies of the +ssDNA genome. For transcription, they the newly created - sense DNA strand and a host RNA polymerase to transcribe + sense mRNA. As shown in Fig. 15, the most important family of ssDNA viruses is:

- **Parvoviruses**, non-enveloped icosahedral viruses that include B19 virus (the cause of "fifth disease," a mild rash in young children), adeno-associated virus (AAV, an apparently fairly harmless virus often considered for gene therapy applications), and parvoviruses that can cause more serious illnesses if you happen to be a dog or a cat.

Group III: Double-stranded RNA viruses must encode their own RNA-directed RNA polymerase, just as other viruses with RNA genomes do, since host cells do not have RNA-directed RNA polymerases. These viruses have little use for the nucleus; dsRNA viruses use their own RNA polymerases both to replicate their dsRNA genomes and also to make mRNA that can be translated within the host cell to produce viral proteins (Fig. 14). Other than having a dsRNA genome and no envelope, these viruses generally follow the replication cycle illustrated in Fig. 18. As shown in Fig. 15, the main virus family of interest that has dsRNA genomes is:

- **Reoviruses**, a very large and very popular family of non-enveloped icosahedral viruses that mostly cause gastrointestinal infections, most of which are also called rotaviruses. They especially love to take vacations on cruise ships.

Group IV: Single-stranded RNA viruses (+ sense) (Fig. 14) also encode their own RNA-directed RNA polymerase, typically replicate outside the nucleus of the host cell, and generally follow the replication cycle illustrated in Fig. 18. There are many families of +ssRNA viruses of major interest (Fig. 15), including:

- **Picornaviruses**, non-enveloped icosahedral viruses that include rhinovirus (the common cold virus, in zillions of different strains for your enjoyment), hepatitis A, and poliomyelitis or polio virus.
- **Astroviruses**, non-enveloped icosahedral viruses that include several gastrointestinal viruses.
- **Caliciviruses**, non-enveloped icosahedral viruses that include hepatitis E and several gastrointestinal viruses.
- **Flaviviruses**, enveloped icosahedral or spherical viruses that include hepatitis C, dengue fever, and several encephalitis viruses (West Nile virus, yellow fever virus, etc.). Hepatitis C is transmitted by blood or sexual contact, but most of the other flaviviruses are transmitted by mosquito bites or tick bites.
- **Togaviruses**, enveloped icosahedral viruses that are famous for their inebriated toga parties, include rubella (German measles) and several encephalitis viruses (multiple types of equine encephalitis, Chikungunya, etc.).
- **Coronaviruses** include the fairly deadly and highly publicized Middle Eastern Respiratory Syndrome (MERS), Severe Acute Respiratory Syndrome (SARS), as well as several mundane respiratory and gastrointestinal viruses that merely inconvenience you.

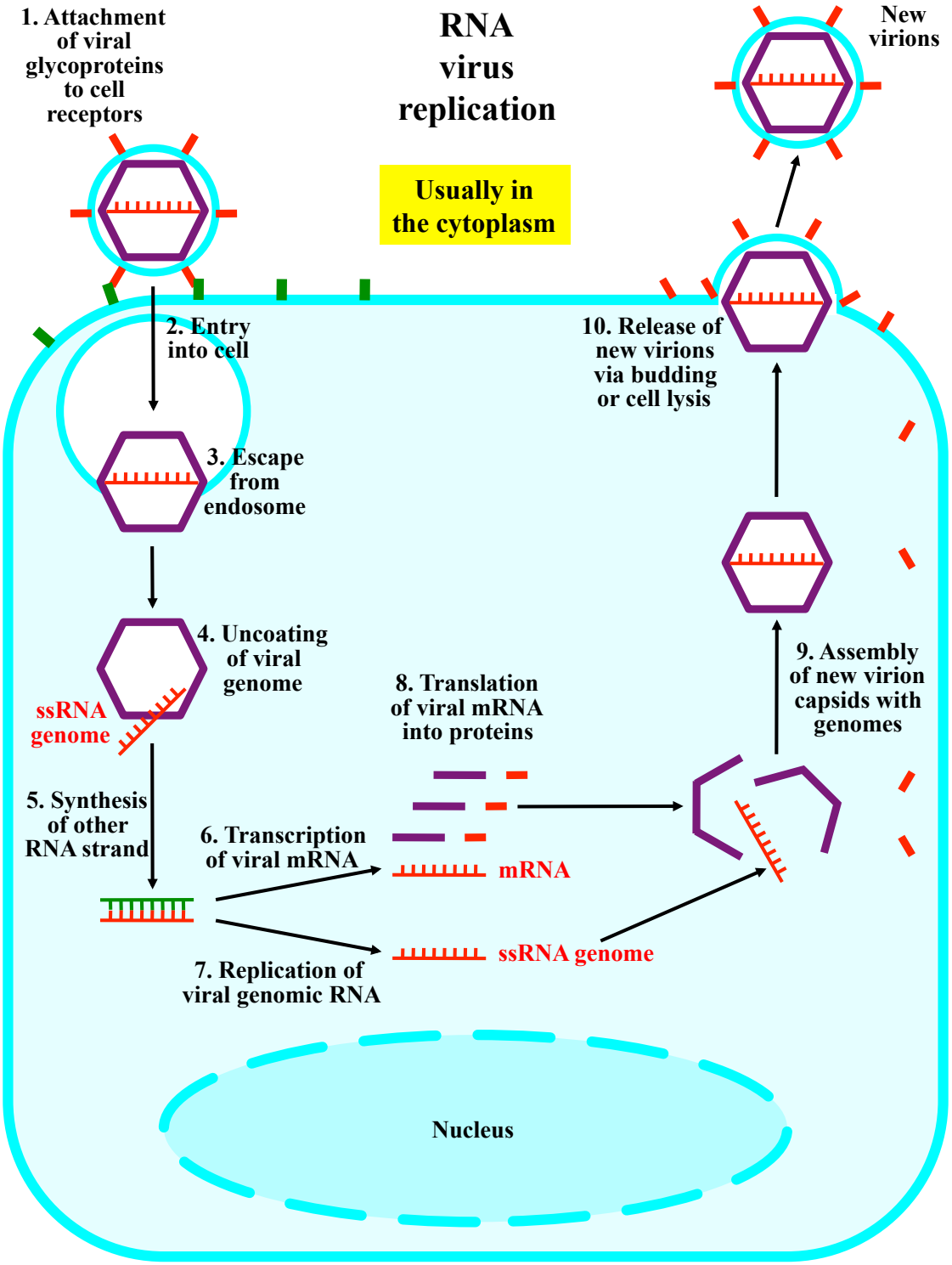


Fig. 18. Replication of a typical RNA virus. Almost all RNA viruses replicate outside the nucleus, since they do not need access to the cell's enzymes for replicating DNA or transcribing DNA to RNA. (Influenza is a major exception–it is an RNA virus but likes the nucleus for sentimental reasons.)

Group V: Single-stranded RNA viruses (- sense) likewise encode their own RNA-directed RNA polymerase (Fig. 14), usually replicate outside the nucleus of the host cell, and generally follow the replication cycle illustrated in Fig. 18. There are many families of -ssRNA viruses of major interest (Fig. 15), including:

- **Orthomyxoviruses**, enveloped eight-segmented viruses that most notably include a zillion strains of influenza (flu) virus. Because influenza carries its genome in eight independent -ssRNA segments, it is easy for two different influenza strains to swap segments if they infect the same cell, leading to the creation of a new influenza strain. Unlike most other RNA viruses, influenza likes to replicate in the nucleus of the host cell.
- **Paramyxoviruses**, enveloped viruses of various shapes, include measles, mumps, respiratory viruses like respiratory syncytial virus, and Hendra and Nipah hemorrhagic fever viruses.
- **Rhabdoviruses**, enveloped bullet-shaped viruses, include rabies (which is transmitted by bites and can cause derangement and death in virtually any mammal, including humans), several other rabies-like lyssaviruses that are not as common or as well understood, and more distantly related viruses that usually do not infect mammals.
- **Arenaviruses**, enveloped viruses with a multiple beaded structure that have a more complicated genome than many of their -ssRNA cousins: the genome is divided into two separate RNA segments, with some regions of them being - sense and some regions being + sense. Arenaviruses include several nasty hemorrhagic fever viruses (Lassa, Junin, etc.) mostly spread by droppings from infected rodents.
- **Bunyaviruses**, enveloped helical viruses with genomes consisting of three -ssRNA segments. Bunyaviruses include hantaviruses (spread by droppings from infected rodents) and other hemorrhagic fever viruses that are spread by insect bites (Rift Valley Fever, etc.).
- **Deltaviruses**, very small enveloped spherical viruses with a circular -ssRNA genome just under 1700 bases, only include hepatitis D. Hepatitis D has so few genes that it can only replicate in cells that are co-infected with hepatitis B virus to provide all the other functions that are essential for replication.
- **Filoviruses**, enveloped thread-like viruses that cause deadly hemorrhagic fever, including several strains of Ebola virus plus its close cousin Marburg virus.

Group VI: RNA viruses that replicate via a DNA intermediate encode a unique reverse transcriptase (RT) that copies the +ssRNA genome to dsDNA (Fig. 14). They insert that dsDNA copy of their viral genes into the host cell's own genome in the nucleus (Fig. 19), then use the host cell's RNA polymerases both to make new copies of their +ssRNA genome and to transcribe mRNAs encoding viral proteins. There is one major family of these viruses (Fig. 15):

- **Retroviruses**, enveloped viruses including human immunodeficiency virus (HIV) and human T-lymphotropic virus (HTLV).

Group VII: DNA viruses that replicate via a RNA intermediate, are just plain weird. They have a perfectly good dsDNA genome, yet insist on using a host RNA polymerase to copy it to RNA, then their own viral reverse transcriptase (RT) to create new dsDNA genome copies from that RNA intermediate. They use host RNA polymerases to make protein-encoding mRNAs from their dsRNA genome (Fig. 14). They can also insert their dsDNA genome into the host cell's own genome in the nucleus (Fig. 19). There is one major family of these viruses (Fig. 15):

- **Hepadnaviruses**, enveloped icosahedral viruses that most notably include hepatitis B.

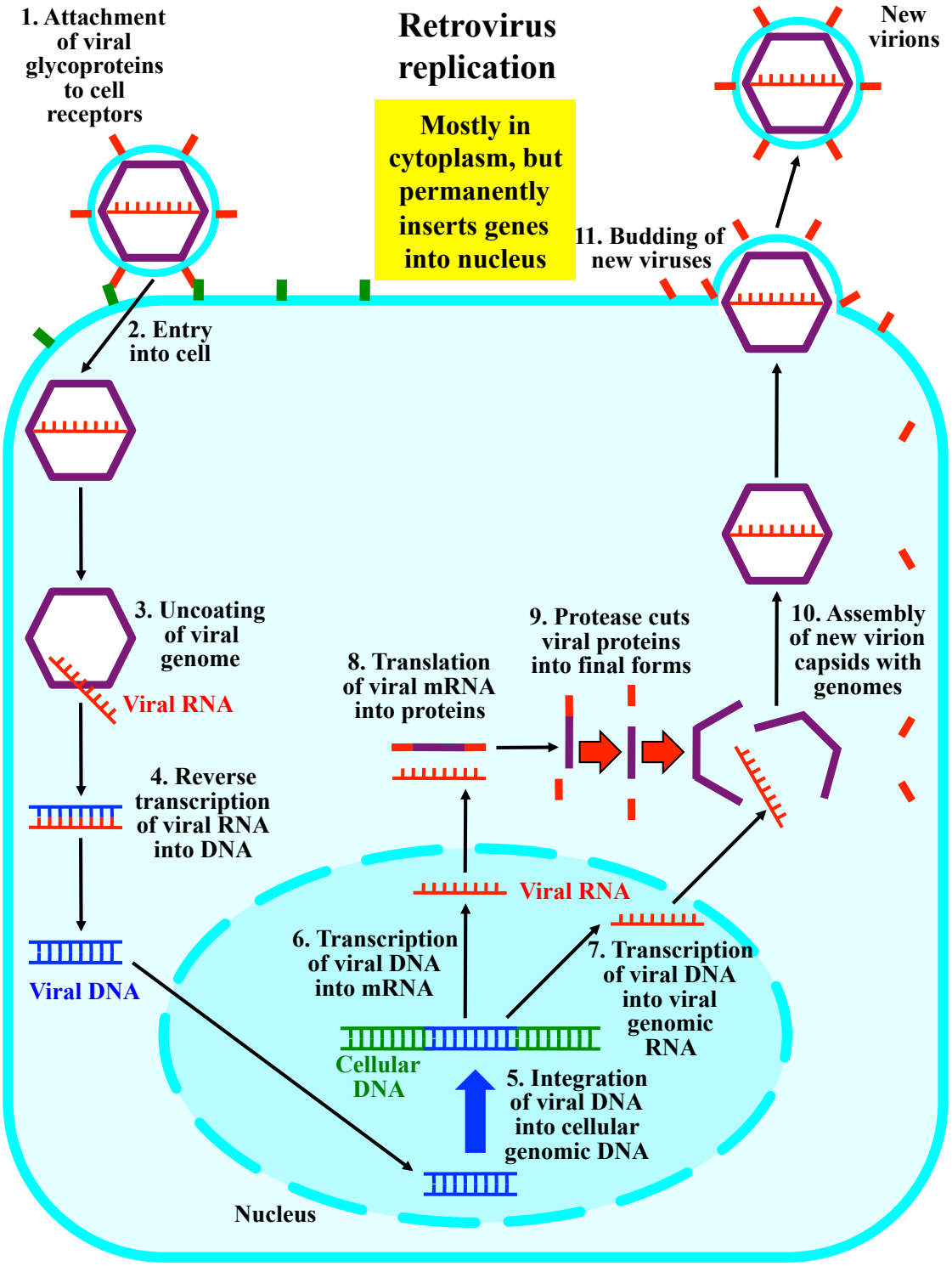


Fig. 19. Replication of a typical retrovirus. Retroviruses convert their viral genome into dsDNA and permanently insert that into the cell's own genome in the nucleus.

Different viruses love to torment you in different ways, as shown in Fig. 20. Some viruses do most of their damage by one of these methods, while other viruses diversify their portfolio and do two or all three kinds of damage:

(a) Some viruses do not kill their host cells, but rather alter host cell functions so that the cell keeps cranking out new copies of the virus, instead of properly performing the cell's original function. Such viruses include:

- Rabies virus has a very specific taste for neurons (brain and nerve cells), and it loves to permanently screw them up but not kill them, which drives you crazy and maximizes the production of more rabies virus.
- Hepatitis B, C, D, and E viruses similarly have a sweet tooth for hepatocytes (liver cells). By altering the function of the liver cells to produce new copies of the virus, these viruses can cause liver cirrhosis and liver cancer.
- Papillomaviruses and polyomaviruses stimulate their host cells to divide, since they want to use host DNA polymerases to replicate their own viral DNA. That uncontrolled cell division can turn the host cells into anything from warts to cancer.
- Herpesviruses latently infect some cells without killing them, usually either neurons or immune system cells, depending on the specific flavor of herpesvirus. Those latently infected cells serve as a base from which the herpesviruses can launch repeated attacks throughout the life of the host, infecting and killing other cell types such as epithelial skin or mucous membrane cells.
- Retroviruses also latently infect some cells without killing them, usually certain types of immune system cells, launching attacks that infect and destroy other host cells.

(b) Most viruses kill their host cells, no negotiations, no surrender. These zealous viruses include:

- Hepatitis A is less concerned about long-term co-option of liver cells than outright killing them, so it tends to run its course much more quickly than other hepatitis viruses. Of course, hepatitis B, C, D, and E viruses do manage to kill a fair number of host cells along the way too. The liver is better at regenerating than other organs, so it becomes a battleground between cellular regeneration and virus infection and killing, sometimes developing cancer in the process.
- Respiratory viruses love to infect and destroy cells in the lining of your respiratory system from your nose to your lungs, which is why respiratory viruses from rhinovirus (the common cold) to influenza (flu) cause many of the symptoms they do.
- Gastrointestinal viruses likewise love to infect and destroy cells in the lining of your gastrointestinal tract from one end to the other, so in the worst case you have stuff coming out of both ends at the same time.
- Human Immunodeficiency Virus (HIV), the most popular retrovirus, outright destroys many cells in addition to latently infecting a few. It is particularly fond of infecting and destroying CD4 or helper T cells, a specific type of white blood cells that help to coordinate the actions of other immune system cells, effectively shutting down most of the immune system (Acquired Immunodeficiency Syndrome or AIDS).
- Hemorrhagic fever viruses infect and destroy many cells, causing damage to the circulatory system, various organs, and sometimes the brain, depending on the specific type of virus.

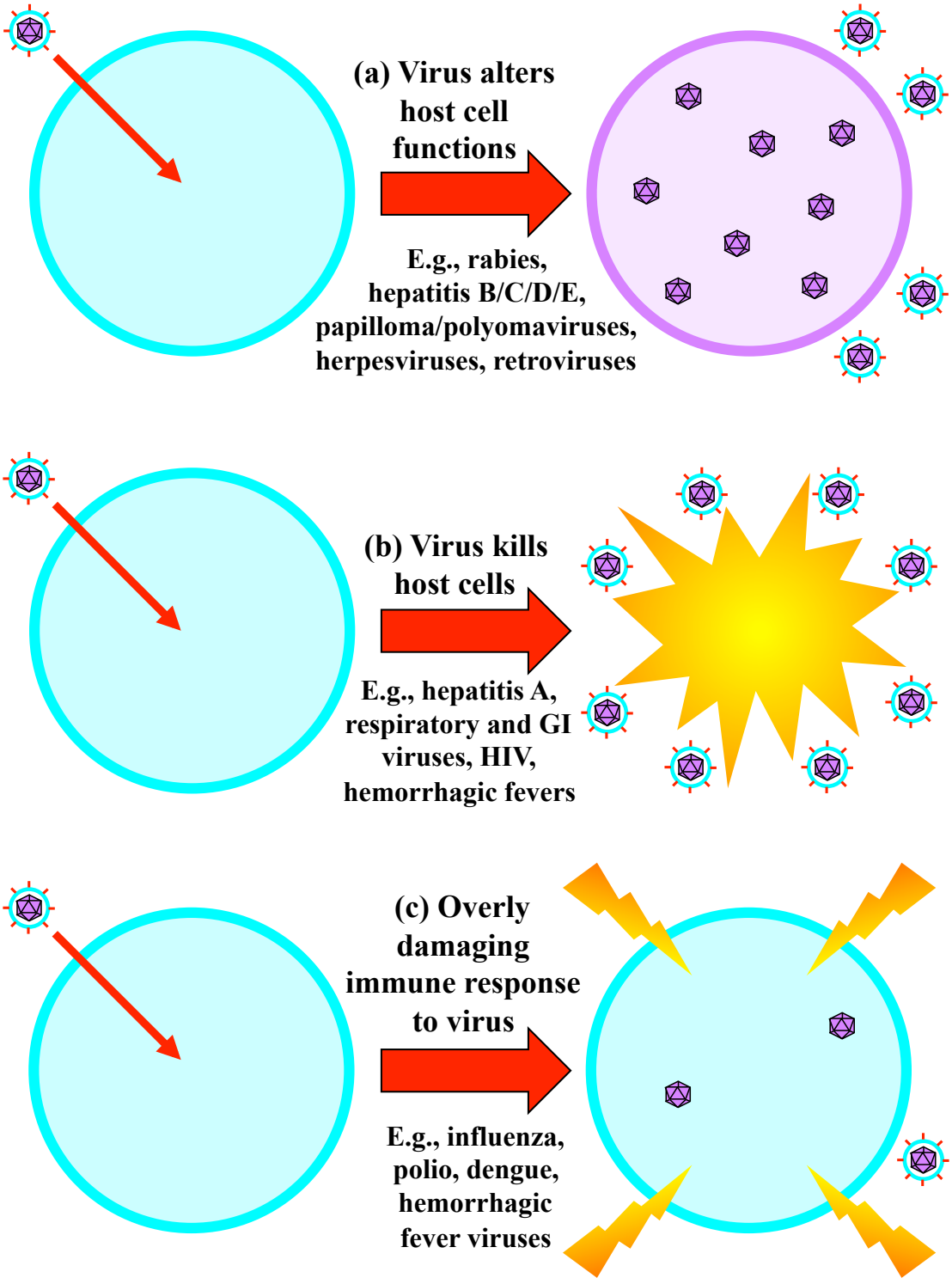


Fig. 20. Viral pathogenesis, or damage caused by a viral infection, may be due to any or all of: **(a)** altering normal cellular functions in virus-infected host cells, **(b)** killing of the infected host cells by the virus, or **(c)** provoking an overly damaging immune response to the virus.

(c) Some viruses do such a great job of provoking and then hiding from the body's immune system that the immune system overreacts in its desperate attempt to clobber the virus, ultimately doing more damage to the body than the virus itself has. Some viruses with this handy feature include:

- Influenza virus can provoke an overreaction from the immune system, in addition to the number of respiratory tract cells that the virus outright kills. Just the sight of influenza virus makes cells start cranking out interferon and other inflammatory cytokines, which gives you that feeling that you have been run over by the school bus, along with strong fever, headaches, muscle aches, and other little joys. The influenza strain that was especially fashionable in 1918 was noteworthy for killing a disproportionately large number of healthy young adults, who died from the "cytokine storm" of their immune system overreaction; young children or older adults with weaker immune systems were less likely to have such a strong response.

- Polio virus looks a little like the natural myelin coating on parts of neurons. When the immune system sees polio virus and learns how to attack it, it also starts attacking the similar looking myelin. That destroys the myelin coating on neurons, which is about as good an idea as chewing the insulation off all the wires in your computer. This adverse response by the immune system leads to nerve damage causing varying degrees of paralysis.

- Dengue virus comes in four major types. If you are infected with any one type, you get sick but then get better, and it usually isn't so bad. If you are re-infected with that same type, your immune system remembers it and rapidly fights if off. However, if you are subsequently infected with any of the other three types of dengue, your immune system recognizes the virus well enough to go to red alert, but not well enough to quickly eliminate the virus. Thus the immune system overreacts, and this overreaction damages lots of cells in the circulatory system, causing hemorrhagic fever that can be fatal.

- Other hemorrhagic fever viruses can likewise provoke an immune system overreaction that causes a great deal of damage to the circulatory system, various organs, and/or the brain, in addition to those cells that the viruses themselves infect and kill.

3.2 Antiviral Therapeutics

Existing antiviral therapeutics can be grouped into categories based on where they intervene in the viral replication cycle, as shown in Fig. 21:

- **Cell attachment inhibitors** block the binding of a specific virus to its specific target receptors on cells it hopes to infect. Currently the main examples of such inhibitors are for HIV.
- **Viral uncoating inhibitors** stop a virus from doing a strip tease and releasing its genes once it gets inside a cell. The best current examples work on influenza, by inhibiting a H^+-sensitive ion channel that lets the flu virus know it has successfully snuck inside a cell via endocytosis.
- **Nucleoside or nucleotide analogues** inhibit viral DNA or RNA synthesis, as shown in more detail in Figs. 22-24. These analogues look just like regular DNA or RNA nucleotides, except they are missing some key part, often part of the back end (3' end). If an unsuspecting virus grabs and adds one of those to its growing DNA or RNA strand, it will be bumfuzzled how to add any further nucleotides to the back end of the analogue. The trick is finding analogues that will fool a specific virus into using them, yet not fool healthy human and animal cells into using them for normal DNA or RNA.
- **Virus-specific antibodies** can be purified from B lymphocytes elsewhere, then injected into people to bind to viruses that are traveling between cells and interfere with their itinerary.
- **Pro-inflammatories** such as interferons to activate the interferon defense pathways and other cytokines to activate the inflammatory defense pathways can be produced and purified elsewhere, then injected into people to heighten their natural antiviral defenses. The downside is they give you that overwhelming feeling of having been run over by a bus, just as your natural pro-inflammatory cytokines do when you start to get sick.
- **Anti-inflammatories** such as ibuprofen or aspirin can block excessive inflammatory responses induced by viruses, which usually doesn't affect the virus much but at least can make you feel better in some cases.
- **Virus-specific vaccines** teach the immune system what to look for, like showing a "wanted" poster to the police (Fig. 7). A good vaccine looks like a certain virus to the immune system, yet does not do any viral mischief itself. Most vaccines use killed versions of the target virus, artificially weakened versions of the target virus, or individual pieces of the target virus.
- **Cell release inhibitors** lock the doors the viruses use to escape infected cells after they have finished replicating inside the cells to make more viruses.
- **Viral protease inhibitors** block protease enzymes that specific viruses use to cut their newly manufactured proteins apart into the final protein components of new virus copies.
- **Retrovirus integrase inhibitors** block the enzymes that retroviruses use to permanently insert their genes into a cell's own DNA.

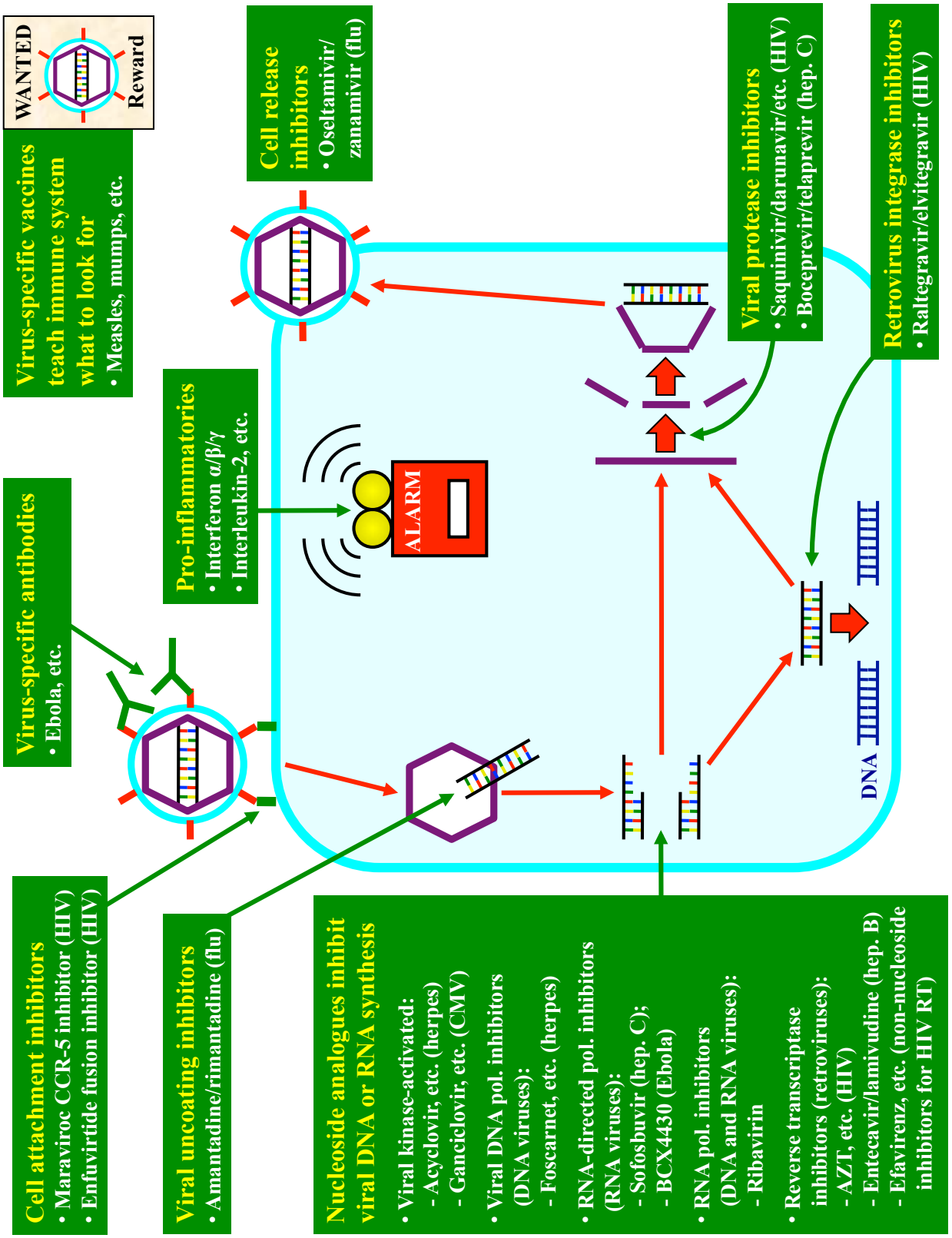


Fig. 21. Major categories of existing antiviral therapeutics.

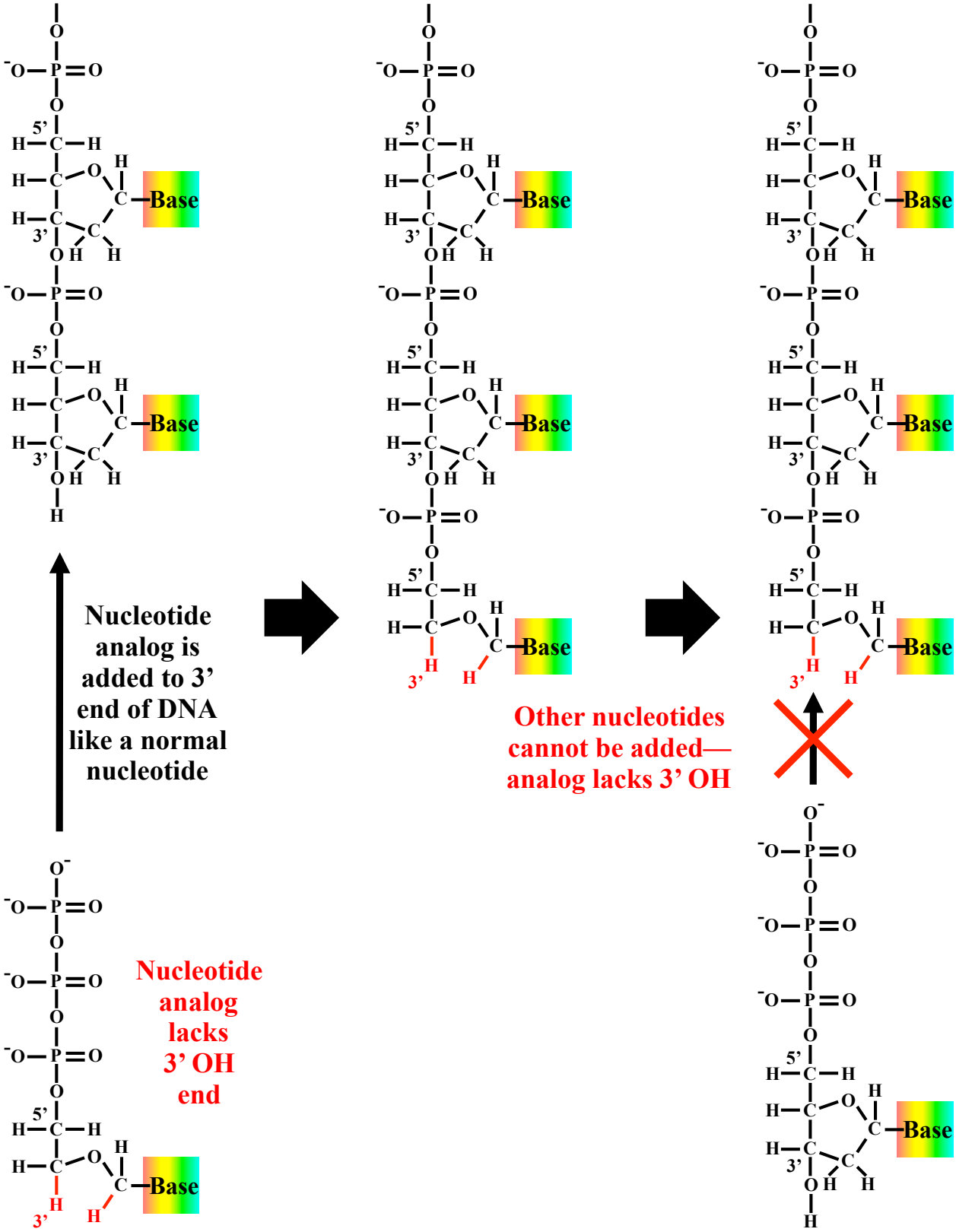


Fig. 22. Incorporation of an analogue blocks extension of that DNA or RNA strand.

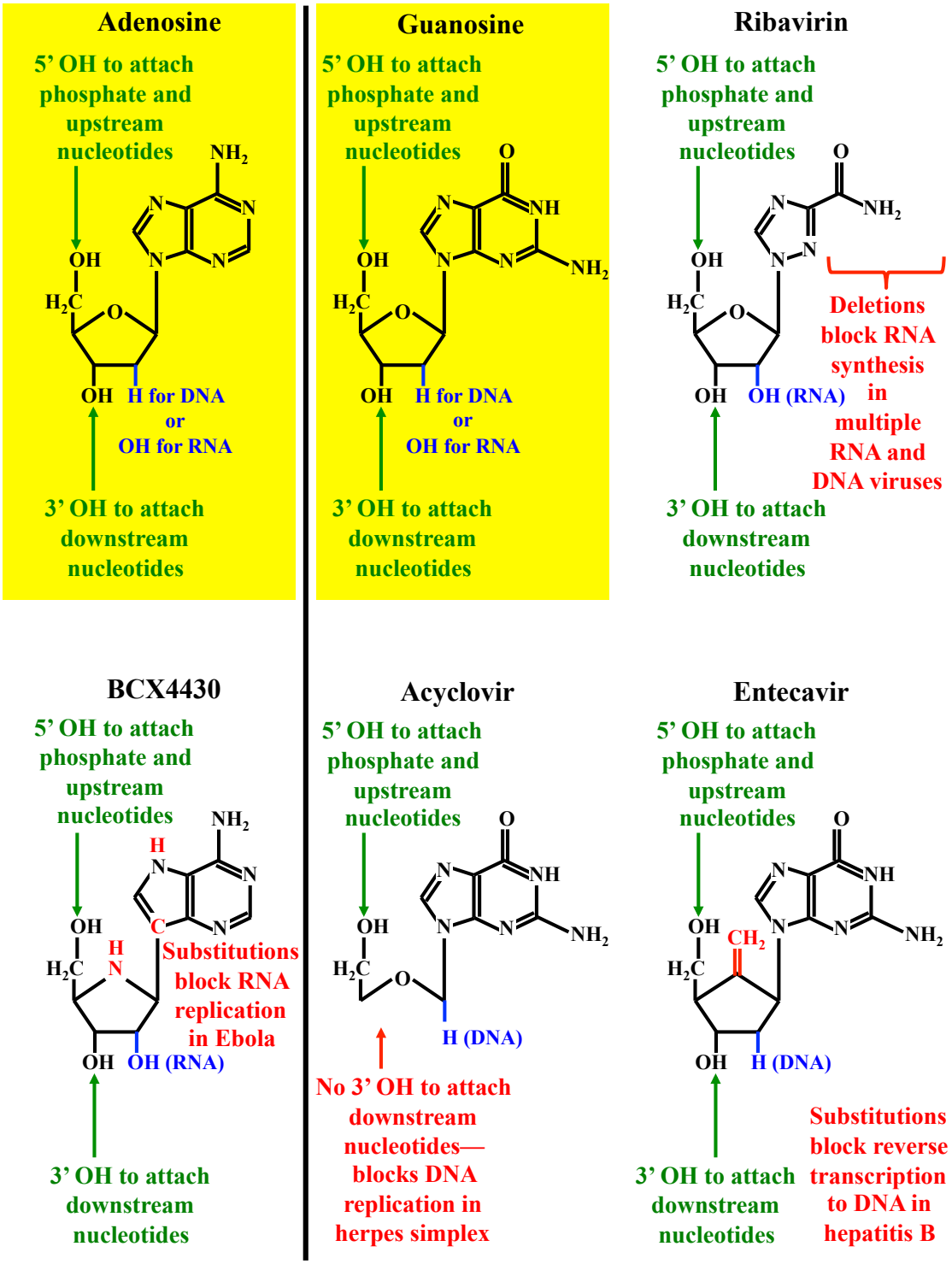


Fig. 23. Examples of specific nucleoside analogues.

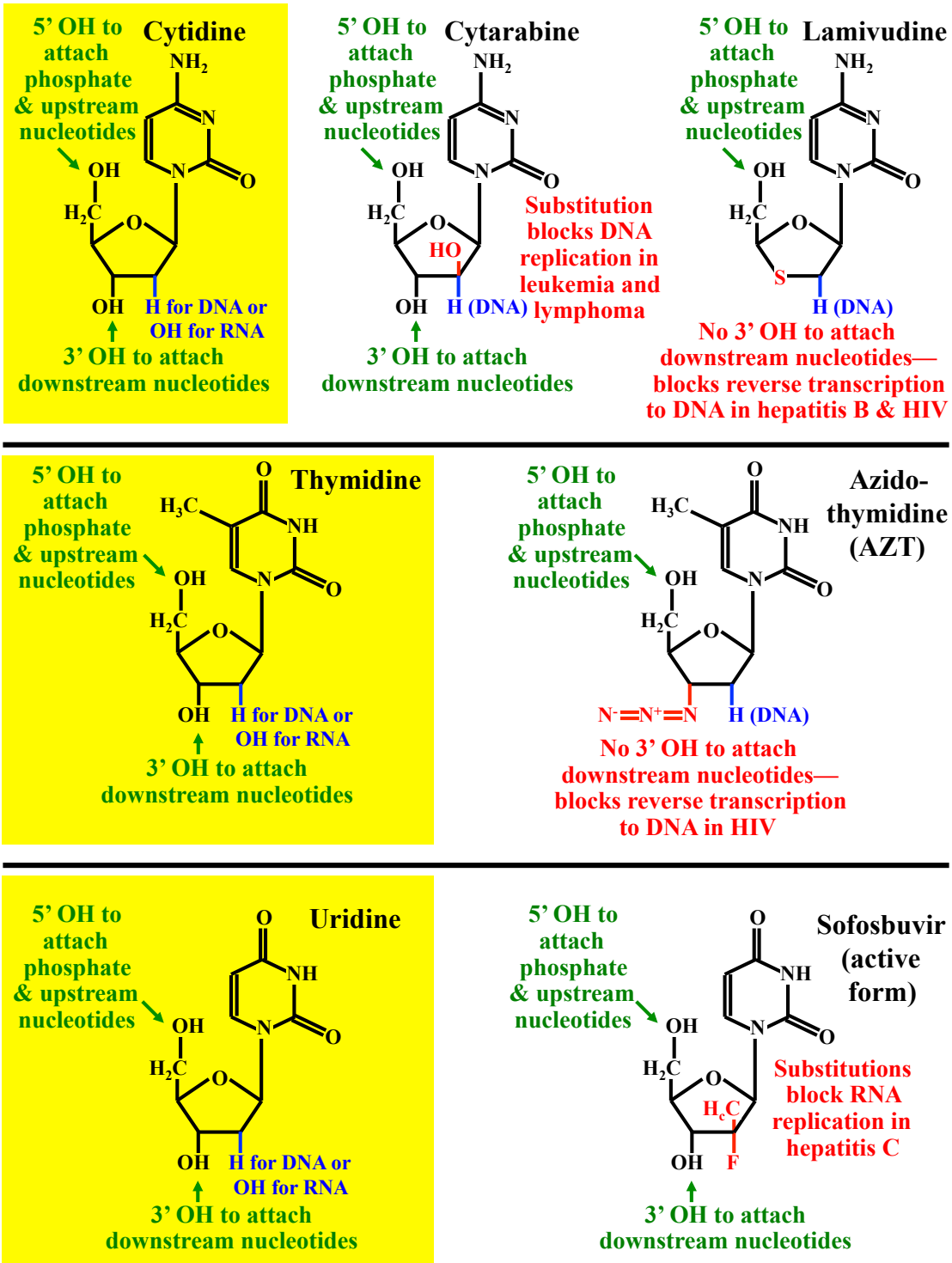


Fig. 24. More examples of specific nucleoside analogues.

3.3 Useful Applications of Viruses

Whereas a number of bacteria naturally make themselves useful, natural viruses generally do not. Nonetheless, biologists with lots of time on their hands have found several ways to adapt certain viruses to use them for practical applications. As shown in Fig. 25, some constructive applications of viruses include:

(a) Treating pathogenic bacteria with bacteriophages. The enemy of your enemy is your friend, so specific bacteriophages (or components derived from them) can destroy certain pathogenic bacterial cells without having any effect on human cells.

(b) Using bacteriophages to display and select proteins, often called **phage display** for short. Different phages are genetically engineered with genes for different proteins added to their coats; thousands of slightly different versions can be produced in parallel. Only phages whose proteins have the desired binding properties stick to a target surface, avoid getting flushed down the drain, and are selected to copy their genes by replicating in bacteria. Thus the biologists can prop up their feet and watch Star Trek reruns while the phages and bacteria do all the hard work of figuring out which of the thousands of initial genes/proteins have the desired property.

(c) Using viruses to deliver genes into cells. There are many noninfectious diseases (everything from cancer to cystic fibrosis) that are caused by defective genes in a person's cells, and that could be cured if we could insert correct copies of those genes into the cells. Although it is very difficult to artificially insert genes into cells in a whole person or animal, viruses are naturally very good at that. Mad scientists have been able to take all of the harmful genes out of a virus and insert beneficial genes, but they are still working on getting the virus to infect the right cells without getting clobbered by the immune system or causing the immune system to freak out and overreact.

(d) Using viruses to kill cancer cells. Some viruses can only replicate in cells that are dividing (and hence have lots of DNA-replicating enzymes), and cancer cells love to divide. Thus one can potentially treat cancer with the right sort of genetically engineered virus, called an oncolytic (cancer splitting) virus, preferentially infecting and killing far more cancer cells than healthy normal cells. Of course, that was also how the killer mutant vampire zombie plague started in that Will Smith *I Am Legend* movie...

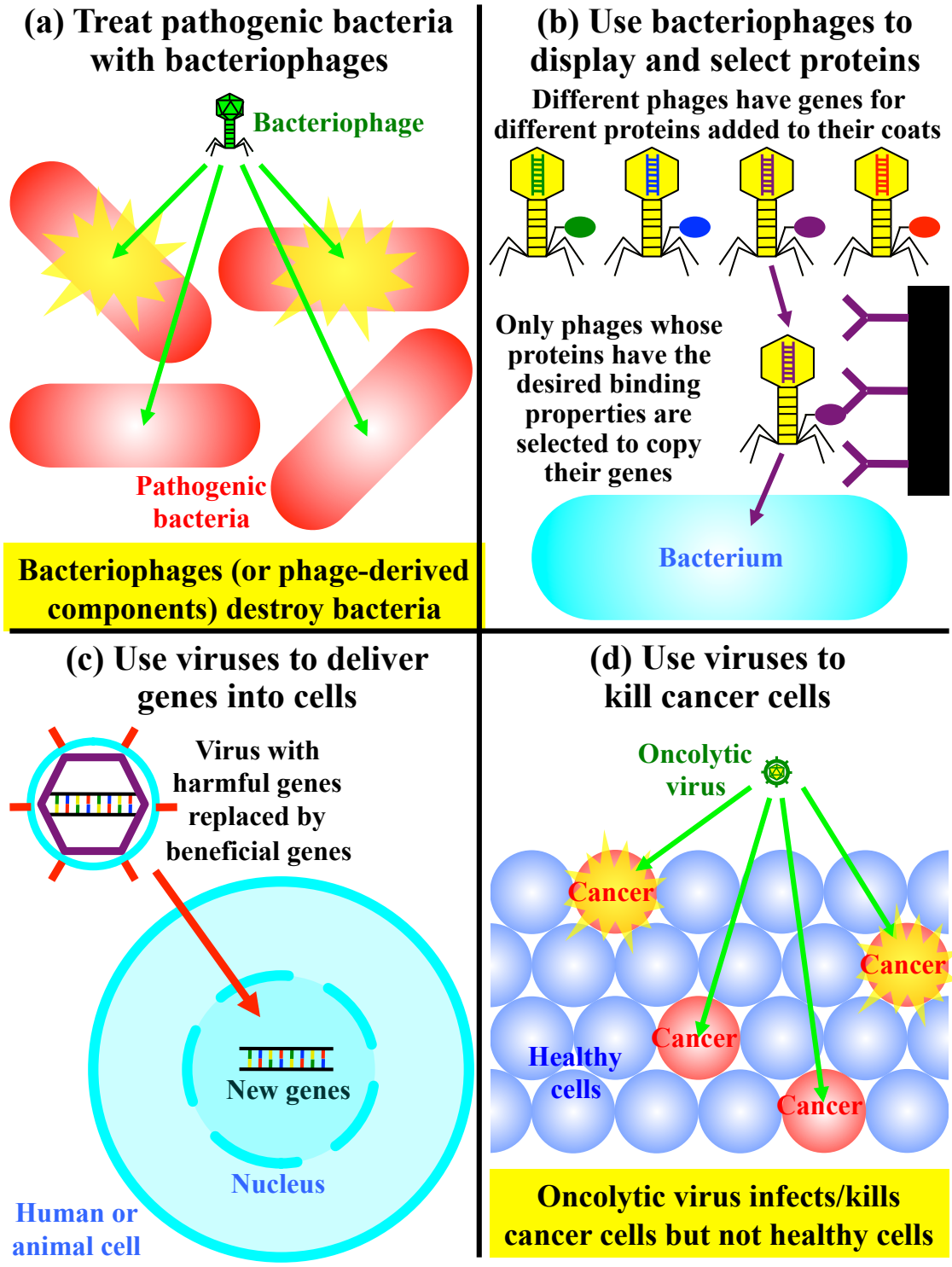


Fig. 25. Some constructive applications of viruses include: (a) treating pathogenic bacteria with bacteriophages, (b) using bacteriophages to display and select proteins, (c) using viruses to deliver genes into cells, and (d) using viruses to kill cancer cells.

4 Bacteria

Bacteria, the next stop on our grand tour of germophobia, are arguably the most widespread and most important microorganisms. This section will cover different types of bacteria and how they work, major classes of antibiotics that can treat bacterial infections, and useful things that are done by some of the good bacteria that aren't trying to kill us.

4.1 Classification and Mechanisms of Bacteria

Figure 26(a) shows the structure of your average garden-variety bacterium or prokaryotic cell. It is awfully darned small, in the range of 0.5-5 μm (microns or millionths of a meter) long, near the limit of what can be readily seen with an optical microscope and roughly 10 times smaller than human and other eukaryotic (complex) cells. Whereas eukaryotic cells are internally divided into many compartmented organelles, most stuff inside prokaryotic cells floats around together in a semi-organized fashion, like many students' rooms. The nucleoid is a large circular DNA chromosome containing most of the bacterial genes, wadded up like a ball of yarn. Many bacteria have one or more plasmids, small circular mini-chromosomes with additional genes that can be easily copied and transferred to other bacteria; sometimes those plasmids teach the bacteria how to be resistant to certain antibiotics or how to make certain toxins. Ribosomes freely floating in the cytoplasm make proteins based on messenger RNA (mRNA) copied from the DNA. Bacteria often save nutrients in inclusion bodies in case they need a midnight snack. And some bacteria have a twirling flagellum that acts like a boat's outboard motor when the bacteria want to swim around and see the sights.

Prokaryotic cells are surrounded by a plasma membrane (like eukaryotic cells), but for extra protection against harsh environmental conditions and/or attack by drugs or a host's immune system, they add a sturdy cell wall and then for some bacteria also a capsule of polysaccharide slime. Different categories of bacteria have different types of cell walls, as illustrated in Fig. 26(b):

- Gram-positive bacteria have a thick peptidoglycan (linked protein + sugar) cell wall with lipoteichoic acid and S-layer glycoproteins thrown in for good measure.
- Gram-negative bacteria have a thinner peptidoglycan cell wall, then another membrane, then an outer layer of lipopolysaccharide.
- Acid-fast bacteria are isolationists and surround themselves with four different cell wall materials: peptidoglycan, arabinogalactan, mycolic acids, and acyl lipids. That creates a very thick waxy cell wall that slows the flow of nutrients into the bacteria (and hence slows their growth) yet gives them great protection against drugs or immune responses that try to kill them. The most important acid-fast bacteria are those that cause tuberculosis and leprosy.
- Archae have a somewhat different plasma membrane, a cell wall of pseudomurein (very similar to peptidoglycan, but without the designer label), and an outer S-layer of glycoproteins. This group of bacteria was largely ignored until recently since most of them are not pathogenic, but they pop up everywhere from soil to deep sea thermal vents.
- *Mycoplasma* bacteria are nudists that actually don't have any sort of cell wall, just a plasma membrane. They like to live inside animal or human cells where they can be very comfortable even without a cell wall.

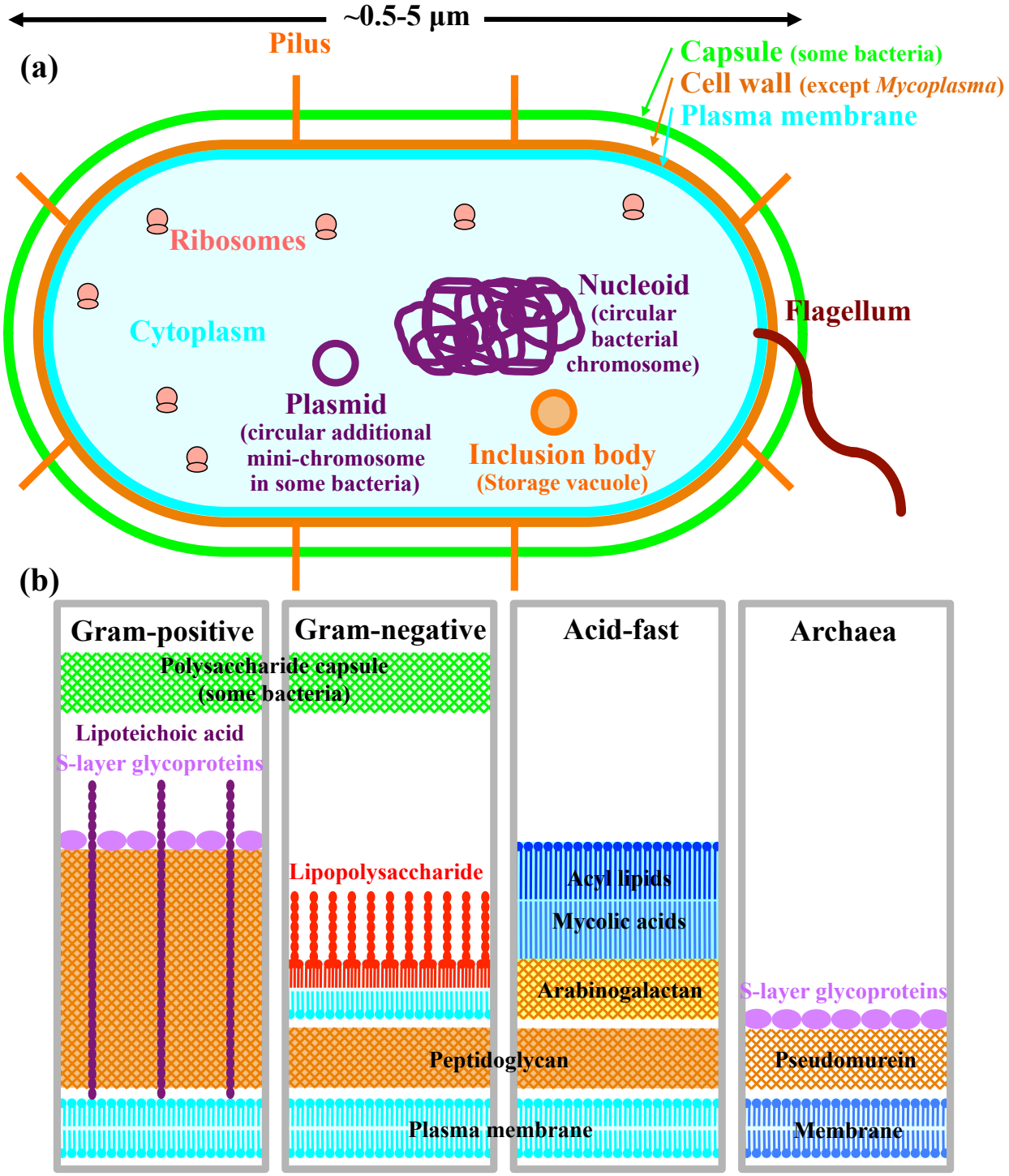


Fig. 26. Typical prokaryotic or bacterial cell, showing (a) the overall structure, and (b) the layers of the cell wall for different families of bacteria. Prokaryotic cells are roughly ~ 10 times smaller than eukaryotic cells. Although they have specialized functions similar to those described for eukaryotic cells, they do not have membranes that divide those different functions into different compartments as in eukaryotic cells. While most prokaryotic cells have fairly similar structures, the major classes of prokaryotes (gram-positive bacteria, gram-negative bacteria, acid-fast bacteria, and archaea) have different types of cell walls.

For a police lineup of many important bacteria, organized both by shape and by cell wall, see Fig. 27. Gram stains and other dyes are commonly used to determine cell wall composition [Figs. 8(c) and 26(b); different dyes penetrate different types of cell walls] and to better visualize the shapes of bacteria under the microscope.

Major cocci or spherical bacteria are mostly Gram-positive and include:

- A few Gram-negative cocci such as *Neisseria*, some species of which can cause bacterial meningitis (*Neisseria meningitidis*) or sexually transmitted gonorrhea (*Neisseria gonorrhoeae*).
- Gram-positive cocci hanging out in pairs (diplococci) surrounded by a polysaccharide capsule. The most important is *Pneumococcus*, a cause of sinus infections.
- Streptococci or long chains of Gram-positive cocci. Some species of *Streptococcus* cause strep throat or sore throat.
- Staphylococci or three-dimensional clumps of Gram-positive cocci that look like clusters of grapes. Some species of *Staphylococcus* can cause staph infections, painful boils on the skin.

Major bacilli or rod-shaped bacteria are mostly Gram-negative and include:

- Gram-negative bacteria that can't quite make up their minds whether to be a coccus or a bacillus (coccobacillus), the most important of which is *Haemophilus*, some species of which can cause bacterial meningitis.
- Gram-negative bacilli such as *Escherichia coli* (*E. coli* to its friends), which includes many peaceful bacteria in your intestine plus a few of their warlike cousins that you hopefully won't encounter.
- Gram-positive bacilli such as the creatively named *Bacillus*, which includes everything from harmless soil bacteria (e.g., *Bacillus subtilis*) to anthrax (*Bacillus anthracis*).
- Acid-fast bacilli, mainly *Mycobacterium tuberculosis* (the cause of tuberculosis, or lung infections that can be very difficult to cure) and *Mycobacterium leprae* (the cause of skin leprosy, thankfully easier to cure).
- Streptobacilli or chains of Gram-negative bacilli, some species of which can cause rat-bite fever (with some assistance from a rat).

Major bacilli or rod-shaped bacteria are mostly Gram-negative and include:

- Comma-shaped Gram-negative bacteria such as *Vibrio*, which includes the species that causes cholera or serious gastrointestinal infections.
- Helix-shaped Gram-negative bacteria such as *Helicobacter*, which causes stomach ulcers.
- Corkscrew-shaped Gram-negative bacteria such as *Borrelia* that causes Lyme disease and *Treponema* that causes syphalis.

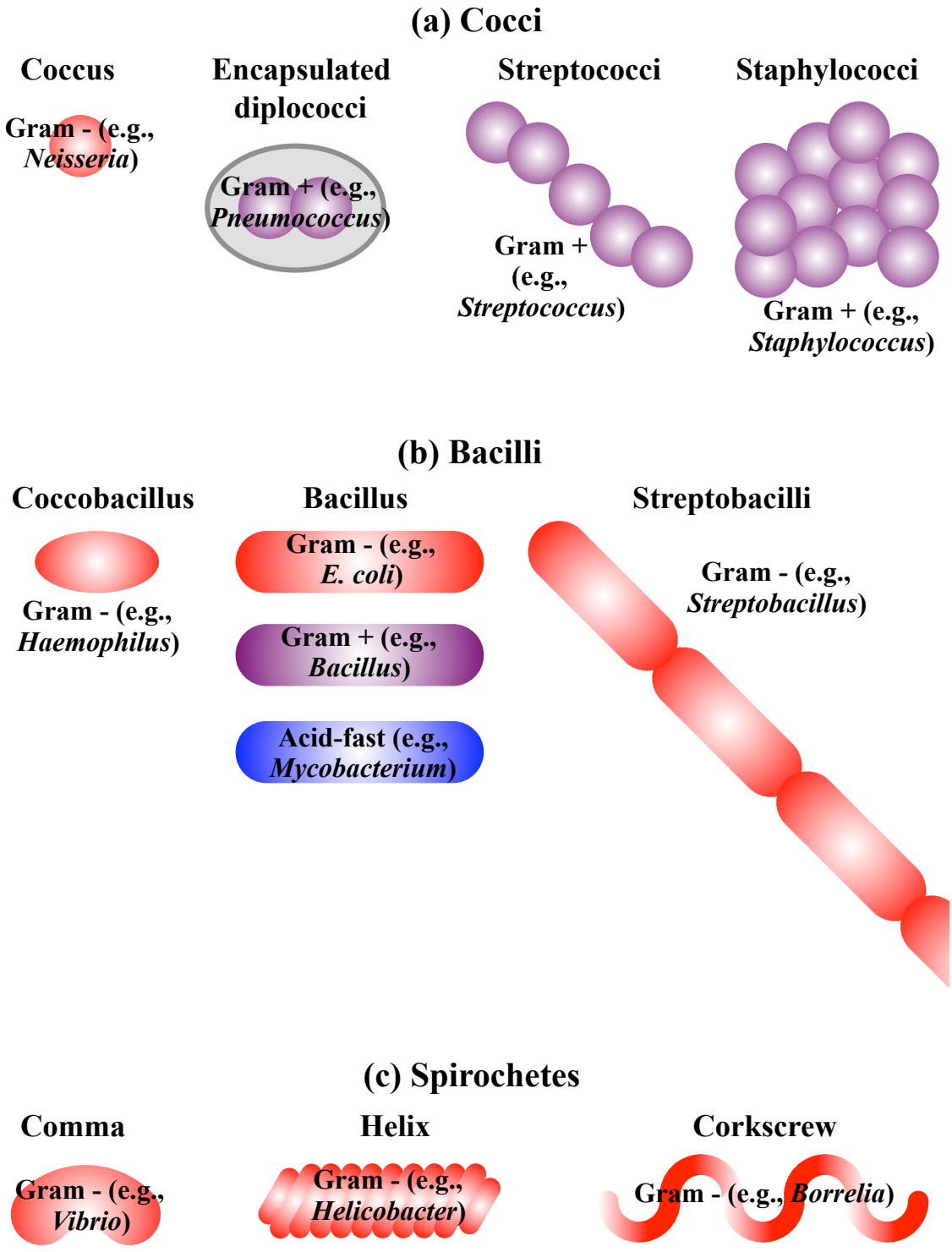


Fig. 27. Categories of bacteria include: **(a)** cocci or round bacteria, **(b)** bacilli or rod-shaped bacteria, and **(c)** spirochetes or spiral-shaped bacteria.

As shown in Fig. 28, major methods of pathogenesis by bacteria such as those listed above include:

(a) Bacteria invading and sickening or killing host cells:

- *Rickettsia* and *Ehrlichia* species are spread by insect bites (ticks, fleas, etc.) and like to live inside endothelial cells lining blood vessels. They replicate in the cytoplasm of their host cells and live off the energy of ATP (adenosine triphosphate) molecules produced by the host cell, instead of producing much of their own ATP. By destroying blood vessel endothelial cells from the inside out, they can cause anything from rashes to headaches to vascular damage.

- *Chlamydia* species are spread by person-to-person contact or in some cases by inhalation, and like to live inside epithelial cells that line mucous membranes. They replicate in the endosomes of their host cells and live off the energy of ATP produced by the host cell, instead of producing any ATP of their own. By destroying mucous membrane cells from the inside out, they can cause anything from potentially very serious urogenital infections (for the sexually transmitted types) to eye infections to respiratory infections, depending on the specific *Chlamydia* species and transmission route.

- *Coxiella burnetii* is spread by inhalation or ingestion of spores from infected livestock such as cattle, sheep, or goats. The spores are very rugged and resistant to killing. Like *Rickettsia* and *Chlamydia*, *Coxiella burnetii* lives off ATP produced inside host cells. It can cause fever, headaches, and pneumonia.

- *Mycoplasma* bacteria lack a cell wall and live inside host cells. Different species can cause anything from respiratory diseases (e.g, *Mycoplasma pneumoniae*) to sexually transmitted diseases (e.g., *Mycoplasma genitalium*).

- *Mycobacterium* species cause tuberculosis (*Mycobacterium tuberculosis*) and leprosy (*Mycobacterium leprae*) and can thrive inside macrophage cells that try to eat them. Their waxy, thick, multilayered cell walls [Fig. 26(b)] make them fairly resistant to destruction by natural responses or by antibiotic drugs, but also impede their intake of nutrients and make them grow very slowly compared to other bacteria.

- *Salmonella* are Gram-negative bacilli with different strains that cause everything from food poisoning to typhoid fever. If ingested, they pass through the intestinal wall and live inside cells in the intestinal lining and inside macrophage cells, causing gastrointestinal illness and potentially spreading to other tissues as well.

- *Listeria* are Gram-positive bacilli; if ingested from contaminated food they can cause illness, especially in pregnant women or people with weakened immune systems. At room temperature in the environment, they use flagella to swim around, but in the human body, they enter macrophage cells and move around inside the cells by polymerizing actin subunits of the cell's own cytoskeleton.

- *Shigella* are Gram-negative bacilli that act like a nasty cross between *Salmonella* and *Listeria*. If ingested, they set up shop inside intestinal wall cells and also in macrophages, and they move around inside the cells by polymerizing actin subunits of the cell's own cytoskeleton.

- *Neisseria* are Gram-negative cocci that live inside host cells. Depending on the *Neisseria* species and the host cell type, they can either cause bacterial meningitis (*Neisseria meningitidis*) or sexually transmitted gonorrhea (*Neisseria gonorrhoeae*).

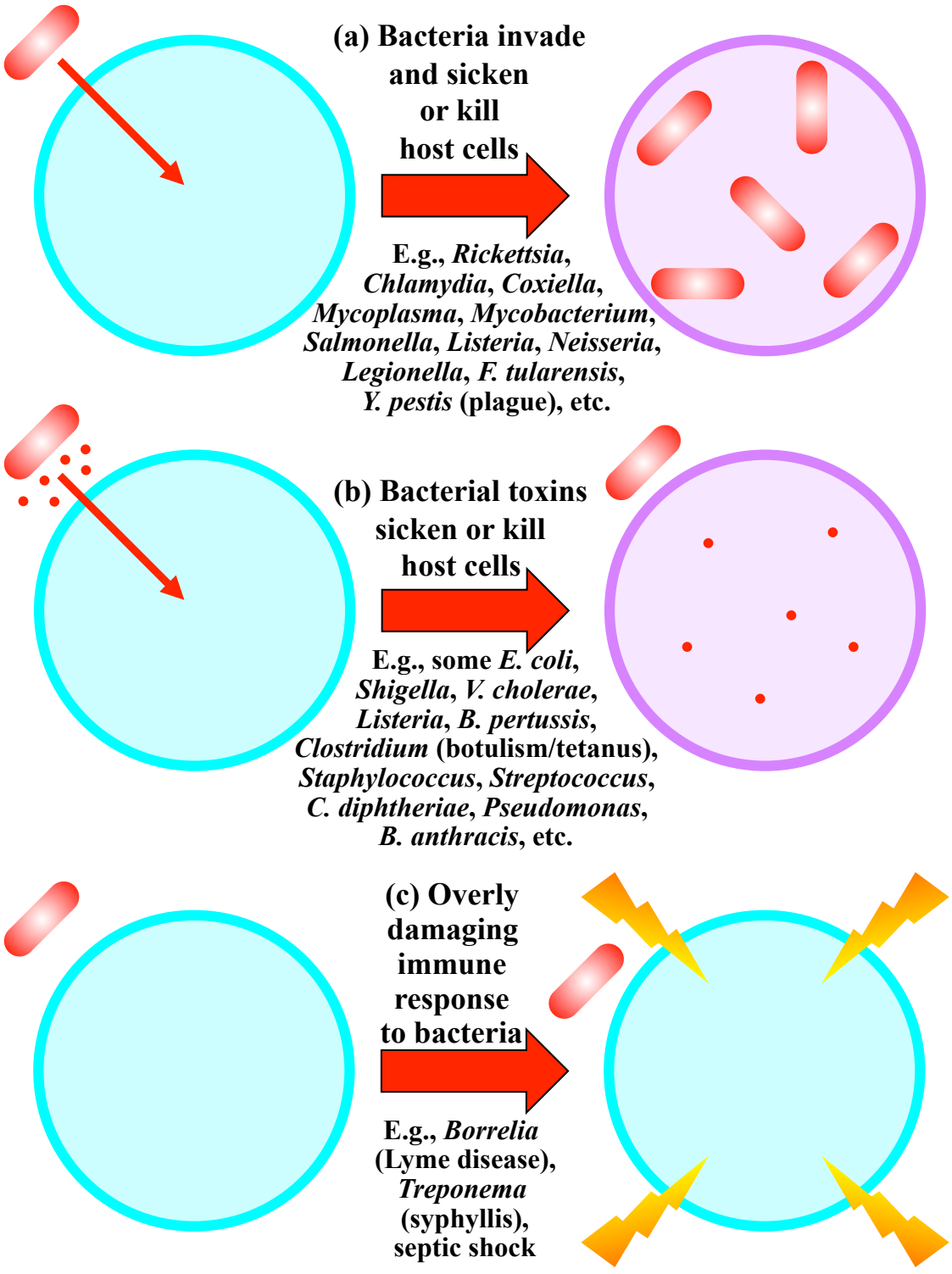


Fig. 28. Major methods of bacterial pathogenesis include: **(a)** bacteria invading and sickening or killing host cells, **(b)** bacterial toxins sickening or killing host cells, and **(c)** an excessive immune response to the bacteria that damages host cells.

- *Legionella pneumophila* can live and replicate inside amoebae (protozoa or single-celled animals to be covered in Section 6.1) in water. If they are aerosolized in sprays of small airborne water droplets from infected water in cooling towers, air conditioning systems, etc., the bacteria can be inhaled by people. Macrophage cells in the lungs eat the bacteria and try to destroy them, but *L. pneumophila* has a variety of tricks that enable it to survive and replicate inside the macrophages. Symptoms can include pneumonia, fever, and headache.

- *Yersinia pestis* bacteria cause plague. They are eaten by macrophage cells in the body, and thrive inside the cells instead of being destroyed there. Bubonic plague is spread by bites from fleas that have fed off infected rats, and especially accumulates inside cells in lymph nodes, making them black and swollen (hence this was sometimes called the "Black Death"). Pneumonic plague is spread by inhalation of bacteria from close contact with other infected hosts, and especially accumulates inside cells in the lungs. Both forms can be quite fatal if not treated promptly.

- *Fransicella tularensis* causes tularemia. It is very similar to *Y. pestis* and its two forms of plague, except the natural host for *F. tularensis* is rabbits.

(b) Bacterial toxins sickening or killing host cells:

- *Shigella* species are Gram-negative bacilli that secrete shiga toxins, which enter human cells (especially kidney cells and neurons) and permanently damage eukaryotic ribosomes to kill the cells, causing renal toxicity and neurotoxicity. Whereas most strains of *E. coli* are non-pathogenic, some pathogenic strains of *E. coli* make shiga toxins too. In fact, it is hard to draw a line between which pathogenic bacteria should be classified as *Shigella* and which should be classified as *E. coli*.

- *Vibrio cholerae* causes cholera or serious gastrointestinal infections. It secretes cholera toxin, which enters cells in the gastrointestinal lining and stimulates G-protein-coupled receptor production of intracellular cyclic adenosine monophosphate (cAMP), making them flood ions and hence water into the intestine, causing massive diarrhea and dehydration.

- *Bordetella pertussis* causes pertussis or whooping cough, an airborne respiratory infection. It secretes pertussis toxin, which enters cells in the lungs and circulatory system and stimulates G-protein-coupled receptor production of intracellular cyclic adenosine monophosphate (cAMP), again making them flood ions and water out of the cells, causing respiratory distress and potentially playing havoc with the immune and endocrine systems.

- *Clostridium* includes several species of anaerobic Gram-positive spore-forming bacilli that love to make toxins in low-oxygen environments inside wounds or inside sealed but contaminated containers of food. *C. botulinum* causes botulism by producing botulinum toxin, which inhibits junctions between nerves and muscles to cause flaccid (limp) paralysis. *C. tetani* causes tetanus by producing tetanus toxin, which activates junctions between nerves and muscles to cause spastic (clenched) paralysis. Other species such as *C. difficile* and *C. perfringens* produce other toxins that kill cells in wounds, causing gangrene.

- *Staphylococcus* includes some species that make staphylococcus enterotoxin B (SEB) or other toxins that insert into cellular plasma membranes and make pores, killing red and white blood cells outright and causing enough ion flow in smooth muscle cells to paralyze them.

- *Streptococcus* includes some species that make streptolysin, another toxin that inserts into cellular plasma membranes and forms pores, killing the cells.

- *Corynebacterium diphtheriae* secretes diptheria toxin, which enters cells (especially heart and liver cells) and inhibits elongation factor 2 (eF2) that is essential for translation of cellular proteins, killing cells and causing serious damage especially to the heart and liver.

- *Pseudomonas* bacteria secrete exotoxin A, which enters cells and also inhibits elongation factor 2 (eF2) and hence translation of cellular proteins.

- *Bacillus anthracis* or anthrax bacteria can live both outside cells and inside macrophage cells. One of their two plasmids (pXO1) encodes two toxins (lethal factor or LF and edema factor of EF) plus a protein that injects the toxins into cells (protective antigen or PA). EF stimulates cAMP to cause edema or swelling of the afflicted tissues, and LF causes cell death. *B. anthracis* spores from infected livestock can be inhaled to cause respiratory infections, ingested to cause gastrointestinal infections, or enter through skin abrasions to cause cutaneous infections.

(c) An excessive immune response to the bacteria that damages host cells:

- *Borrelia*, the spiral-shaped Gram-negative bacteria that cause Lyme disease, are transmitted by tick bites, often create a bull's-eye-shaped area of inflammation at the bite site, then travel throughout the body and successfully avoid being clobbered by the immune system. However, the immune system keeps trying to target the bacteria and inappropriately attacks somewhat similar looking normal host molecules in joints and neurons, which can lead to persistent arthritis-like and multiple-sclerosis-like autoimmune disease even if the bacteria are wiped out by antibiotics.

- *Treponema* are also spiral-shaped Gram-negative bacteria that can persist indefinitely in the body and provoke the immune system to cause autoimmune diseases, but *Treponema* bacteria are transmitted sexually and also mother-to-child. Their associated disease is syphilis, which if untreated can ultimately cause neurological damage, cardiac damage, and tumor-like gummas or balls of inflamed tissue almost anywhere in the body.

- Most bacteria, however harmless, or even components of bacteria, will provoke an extreme overreaction by the immune system if they are released into the bloodstream; this is called sepsis or septic shock, and most commonly occurs if injury to the gastrointestinal tract allows bacteria normally found there to enter the bloodstream. Cells react by releasing massive amounts of inflammatory cytokines, which can cause rapid death if not treated promptly. Generally the bacterial components that provoke the strongest inflammatory responses are components of the bacterial cell wall, since those are usually all the body sees of an intact bacterial cell. Sometimes the cell wall components of bacteria are called endotoxins because they are so provocative.

4.2 Antibacterial Therapeutics

In creating antibacterial therapeutics or antibiotics, the challenge is to find drugs that will clobber bacterial cells but not human cells. The solution is to find something different that bacterial cells have but human cells do not, then create drugs that block that bacterial something. (The same general idea is used to target other classes of pathogens while minimizing any side effects to the human host.) As illustrated in Fig. 29, antibiotics can be divided into groups, based on what targets they attack in bacterial cells.

One major difference between bacterial and human cells is that bacteria have a cell wall around their plasma membrane (Fig. 26), whereas human cells only have a plasma membrane. As shown in Fig. 30, cells are sensitive to osmosis, or diffusion of water across the plasma membrane from whichever side has fewer dissolved molecules (and hence more water molecules) to the side that has more dissolved molecules (and hence fewer water molecules). If a cell only has a plasma membrane, it can easily explode or implode if the concentration of dissolved molecules outside the cell differs from that inside the cell. Human cells are naturally quite concerned about that and consequently very OCD about keeping the concentrations well balanced. On the other hand, the thick cell walls of bacteria help to protect them from explosions or implosions, so they don't pay as much attention to whether their internal and external concentrations stay balanced. If a drug interferes with bacterial cell walls, the drug will have no effect on human cells, yet it will leave cocky bacterial cells vulnerable to explosion or implosion when they are floating in molecule concentrations that they would otherwise have simply sneered at.

Therefore, many antibiotics attack the bacterial cell wall because it is just so darned tempting. The structures of these drugs generally mimic part of the structure of the bacterial cell wall components such as peptidoglycan, so they get taken up by the cell wall synthesizing enzymes (such as transpeptidases), bind irreversibly to the enzymes or to the incomplete cell wall, and prevent the cell wall from being finished. Some major types of antibiotics that target the cell wall include:

- β-lactams (penicillins [penicillin, amoxicillin, ampicillin, etc.], cephalosporins [cefazolin, cefalexin, cefixime, etc.], carbapenems [e.g., ertapenem], monobactams [e.g., aztreonam])
- Glycopeptides (vancomycin, telavancin, teicoplanin, etc.)
- Bacitracin (blocks transport of peptidoglycan components)
- Cycloserine
- Fosfomycin

β-lactamase inhibitors block bacterial β-lactamase enzymes that would otherwise destroy β-lactam antibiotics, so they are often mixed in with β-lactam antibiotics. β-lactamase inhibitors include:

- Clavulanate
- Sulbactam
- Tazobactam
- Avibactam

Antibiotics that target unique features in bacterial vs. animal cell plasma membranes include:

- Polymyxins
- Cyclic lipopeptides (e.g., daptomycin)

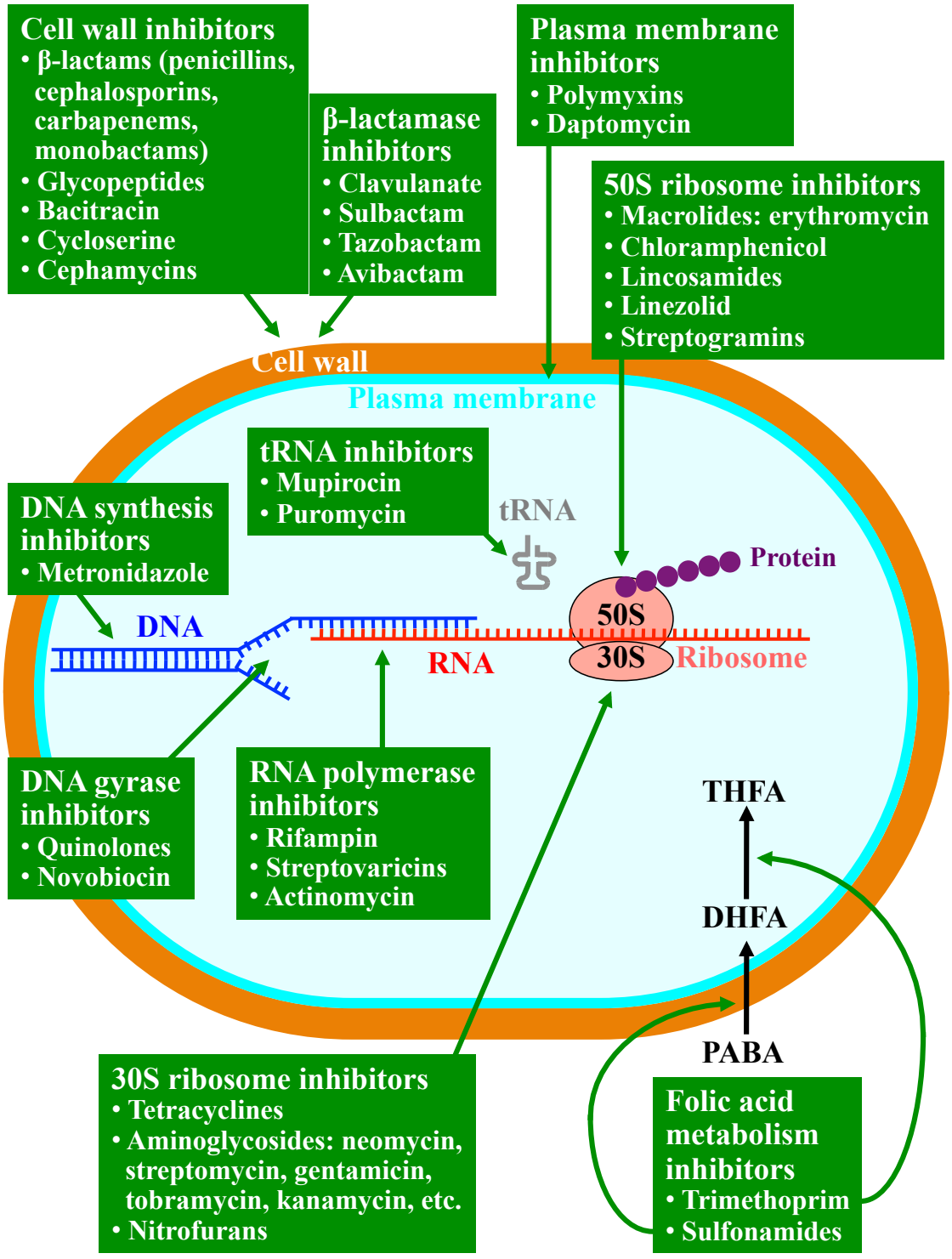


Fig. 29. Different categories of antibiotics attack different features that distinguish prokaryotic cells from eukaryotic cells.

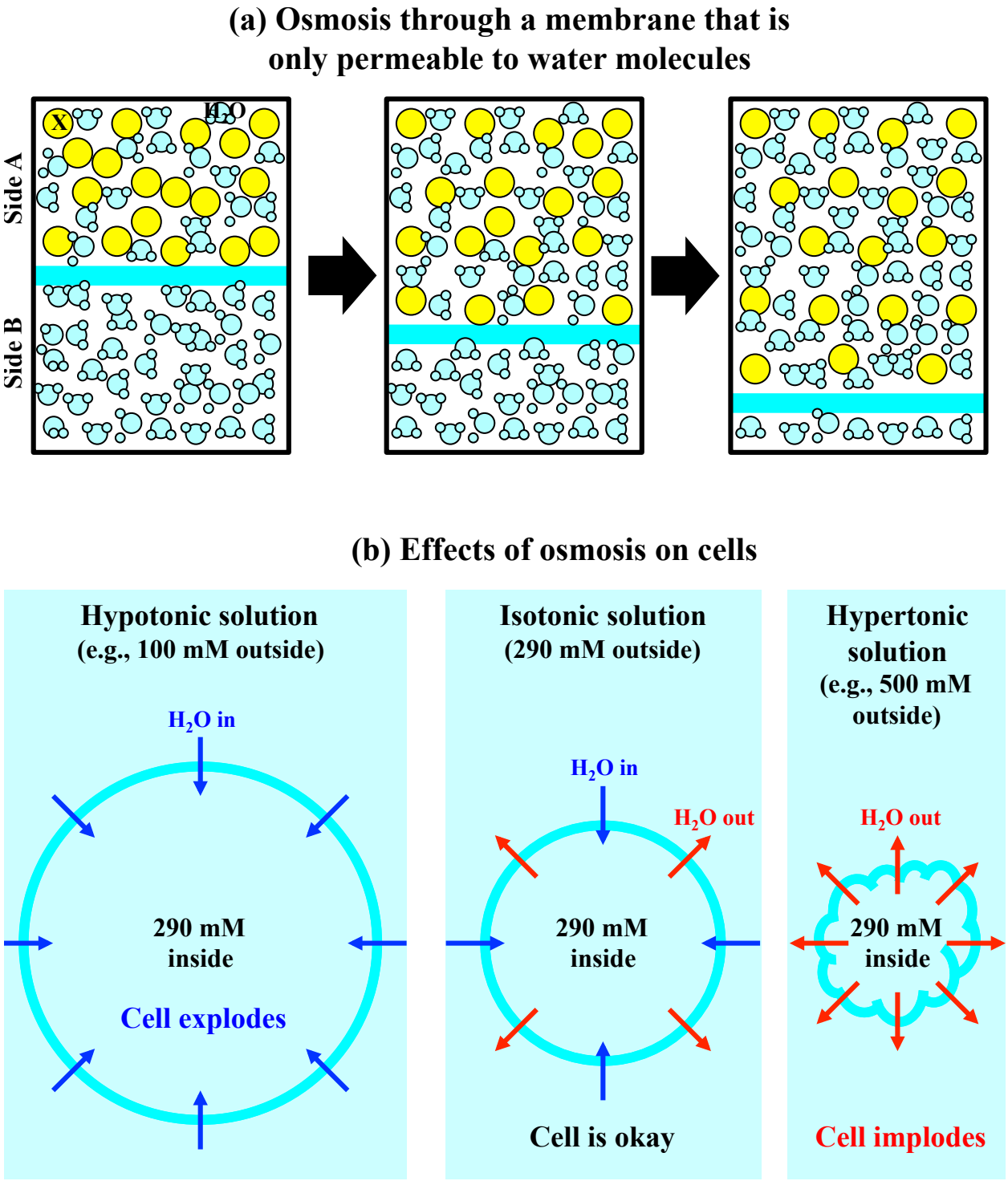


Fig. 30. Osmosis. **(a)** Osmosis, or diffusion of water molecules across a membrane that is permeable to water but not to X molecules, which have a higher concentration on one side. **(b)** Effects of osmosis on cells placed in **hypotonic** (lower concentration of dissolved molecules outside than inside the cell), **isotonic** (same concentration of dissolved molecules), and **hypertonic** (higher concentration of dissolved molecules outside than inside the cell) solutions.

Another major difference between bacterial and human cells is their ribosomes. Both cell types use ribosomes to produce proteins, and the ribosomes are fairly similar, as shown in Fig. 31. However, there are important differences between bacterial (prokaryotic) and human or animal (eukaryotic) ribosomes. Thus it is possible to create drugs that will bind to bacterial ribosomes and stop their protein production, yet not affect the ribosomes or protein production in human cells. Of course, if you were an evil bacterium out for world domination, you might want to do it the other way around.

The large ribosomal subunit is composed of 31 proteins and 2 ribosomal RNAs (rRNAs) in prokaryotes, but 50 proteins and 3 rRNAs in eukaryotes. Many antibiotics attack these key features that make the prokaryotic large (50S) ribosomal subunit different from the eukaryotic large ribosomal subunit, thereby inhibiting bacterial protein synthesis:

- Macrolides (erythromycin, azithromycin, clarithromycin, etc.)
- Chloramphenicol
- Lincosamides (e.g., lincomycin, clindamycin)
- Oxazolidinones (e.g., linezolid)
- Streptogramins

Likewise, the small ribosomal subunit is composed of 21 proteins and 1 rRNA in prokaryotes, but 33 proteins and a larger rRNA in eukaryotes. A number of antibiotics attack these key differences in the prokaryotic small (30S) ribosomal subunit, again inhibiting bacterial protein synthesis:

- Tetracyclines (tetracycline, doxycycline, etc.)
- Aminoglycosides (neomycin, streptomycin, gentamicin, tobramycin, kanamycin, etc.)
- Nitrofurans (e.g., furazolidone, nitrofurantoin)

In a similar fashion, some antibiotics selectively inhibit prokaryotic but not eukaryotic transfer RNAs (tRNAs), including:

- Mupirocin
- Furanomycin

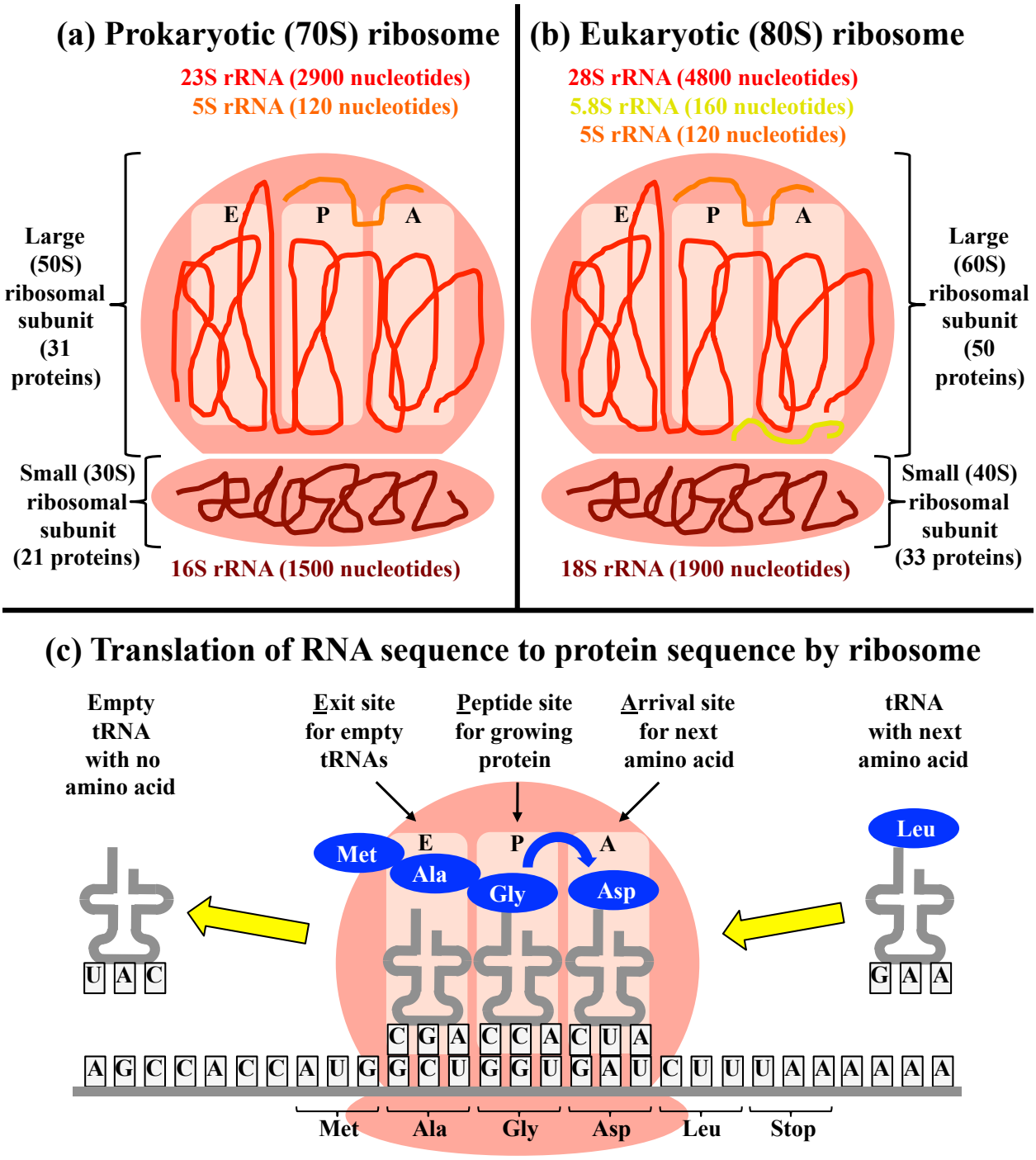


Fig. 31. Prokaryotic vs. eukaryotic ribosomes. **(a)** Prokaryotic ribosomes are composed of a large subunit (which contains two ribosomal RNAs or rRNAs and 31 proteins) and a small subunit (which contains one rRNA and 21 proteins). **(b)** Eukaryotic ribosomes are composed of a large subunit (which contains three rRNAs and 50 proteins) and a small subunit (which contains one rRNA and 33 proteins). **(c)** Despite their differences in size, prokyarotic and eukaryotic ribosomes work in a similar fashion to create proteins by translating a messenger RNA (mRNA) sequence into the amino acid sequence of the corresponding protein. Transfer RNAs (tRNAs) bring each new amino acid to the ribosome.

As shown in Fig. 29, some antibiotics selectively target prokaryotic DNA synthesis enzymes:

- Metronidazole

Similarly, antibiotics that inhibit prokaryotic but not eukaryotic DNA gyrases (Fig. 29) include:

- Quinolones (ciprofloxacin, levofloxacin, etc.)

Some antibiotics inhibit prokaryotic RNA polymerases but not eukaryotic RNA polymerases:

- Rifampin
- Streptovaricins
- Actinomycin

Finally, some antibiotics inhibit prokaryotic enzymes involved in essential folic acid synthesis:

- Trimethoprim
- Sulfonamides (sulfadimethoxine, sulfamethizole, etc.)

That would be a happy end to the story, except some bacteria have learned to fight back and become resistant to certain types of antibiotics. Figure 32 shows some of the major mechanisms by which bacteria can resist antibiotics:

(a) In some cases, bacteria can decrease the permeability of their cell wall or its channels to antibiotics, so that antibiotics no longer penetrate into the bacteria. Some bacteria use that method against vancomycin and β-lactams.

(b) In other cases, bacteria actually use pumps to spit out any antibiotic that gets into them. Some bacteria can use that method against tetracyclines, quinolones, aminoglycosides, β-lactams, and macrolides.

(c) A variety of bacterial enzymes can interfere with or destroy antibiotics. Some bacteria can use that method against β-lactams, aminoglycosides, macrolides, rifamycins, chloramphenicol, tetracyclines, and vancomycin.

(d) Finally, bacteria can mutate to slightly modify the shape of a target an antibiotic is designed for, so that the antibiotic will no longer attack that target. Some bacteria can use that method against macrolides, quinolones, aminoglycosides, penicillins, vancomycin, and rifamycins.

Antibiotic resistance is why it is so important to take a full course of antibiotics and not just stop as soon as you start to feel better. As illustrated in Fig. 33, the first doses of an antibiotic will wipe out most of the bacteria, but any mutant bacteria that are slightly resistant to the antibiotic will last longer. If you stop taking antibiotic then, those slightly resistant bacteria will have babies, and all of the resulting bacteria will be slightly resistant to that antibiotic. After a few more rounds of taking a little of the antibiotic but not enough, the bacteria may have found more mutations that will make them highly resistant to the antibiotic. In contrast, if you take the complete course of antibiotic without a break, eventually even the slightly resistant bacteria keel over before they can give rise to more resistant offspring.

For really wily bacteria that can mutate very easily (such as tuberculosis), doctors usually prescribe a cocktail or mixture of several antibiotics. That way even if the bacteria try to mutate to become resistant to one of the antibiotics, they will still get clobbered by the other antibiotics in the cocktail.

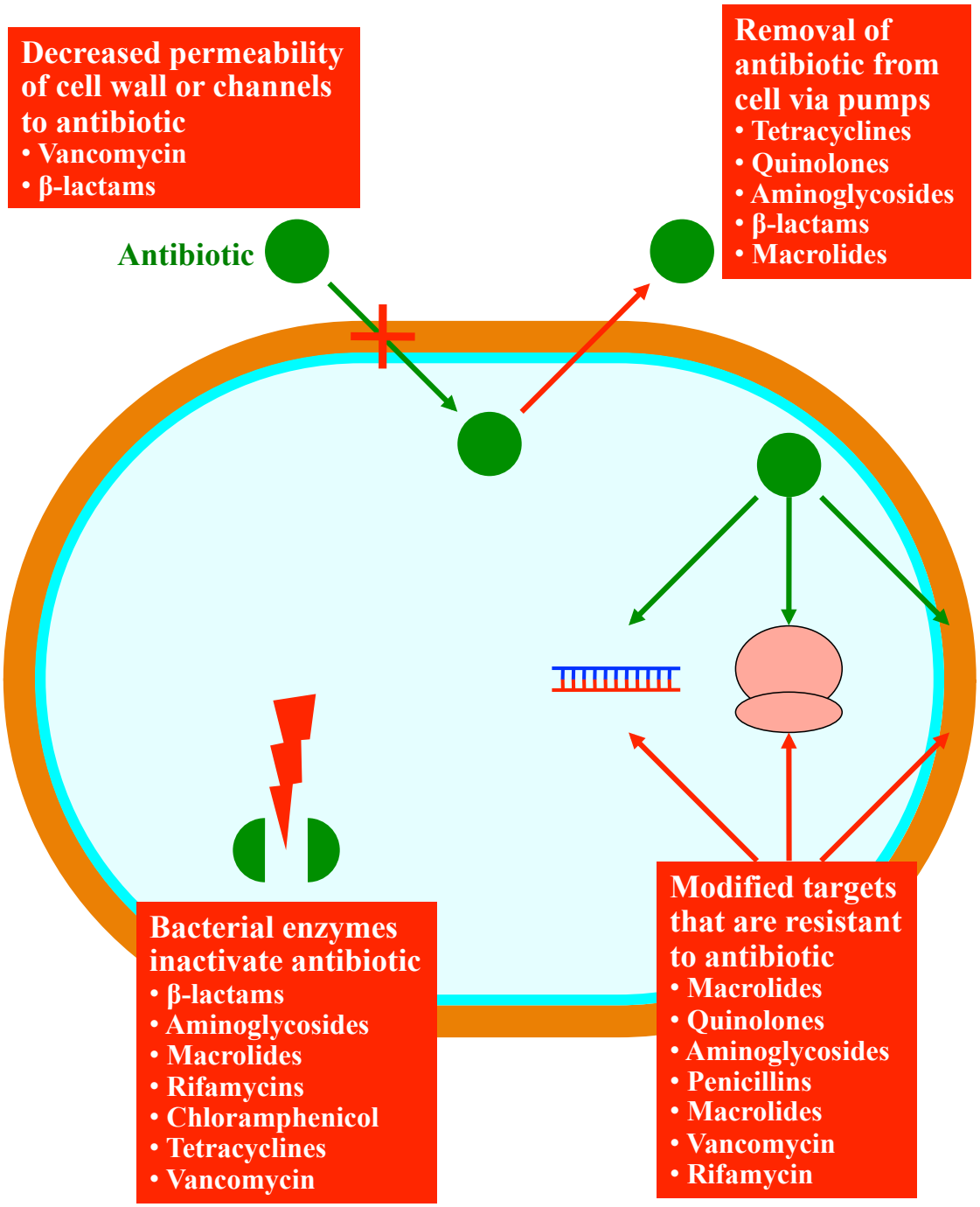


Fig. 32. Mechanisms of antibiotic resistance in bacteria.

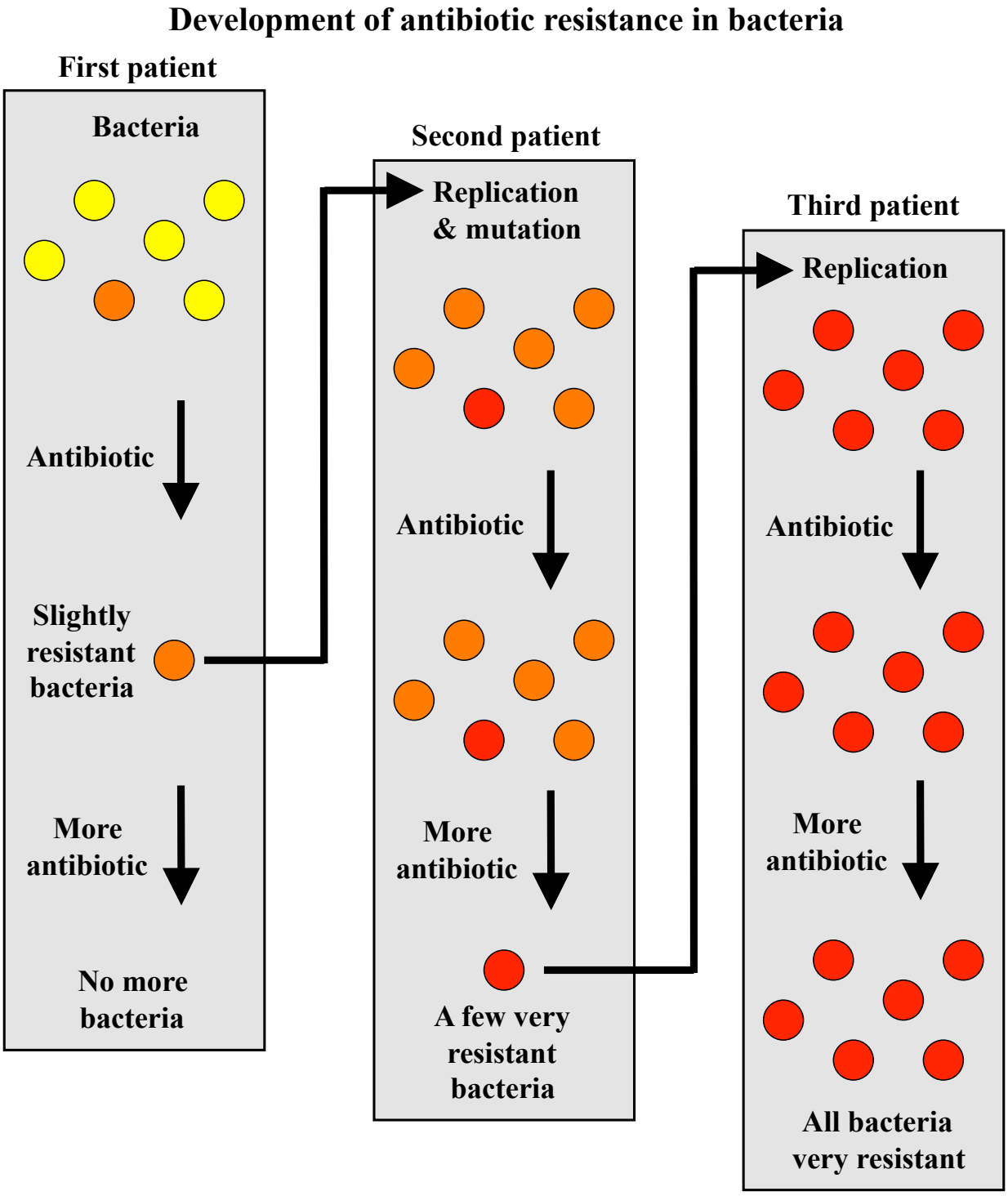


Fig. 33. Development of antibiotic resistance in bacteria.

4.3 Useful Applications of Bacteria

Not all bacteria are bloodthirsty little buggers out to kill you. Many leave you alone, and some are downright obsequious. Figure 34 shows examples of useful bacteria, including:

(a) Normal human microbiota. Your body actually contains about 10 times more bacterial cells than human cells, mostly on your skin and in your gastrointestinal tract. In fact, we are so outnumbered, we are lucky that the little guys don't take a vote to do away with us entirely. The relationship is mutually beneficial though, since we provide them with free room and board, and by occupying all of that real estate they make it harder for really nasty bacteria to set up shop. Bacteria in the gastrointestinal tract aid with digestion, and they may aid (or in some cases hinder) the immune and endocrine systems.

(b) Nitrogen fixation is not being obsessed with all the nitrogen in the air around us. Rather, it is when bacteria in the soil or water absorb nitrogen gas (N_2) from the air and convert it into ammonia (NH_3) or other nitrogen-containing molecules that can be readily taken up by plants and used to build larger biomolecules. Without that, there would be no plants, or animals that eat plants, or animals that eat animals that eat plants, or humans that eat fast food.

(c) Making yogurt, cheese, etc. Milk can be turned into yogurt, a variety of different cheeses, and other products by adding specific types of bacteria and treating the mixture under the right sort of conditions. In general, the bacteria convert lactose (milk sugar) to glucose (simple sugar), then use fermentation to convert that glucose to lactic acid or lactate, as illustrated in Fig. 35(a). Some bacteria can also ferment sugar to ethanol or ethyl alcohol plus carbon dioxide, as shown in Fig. 35(b), but usually certain species of yeast (fungi) get the pleasure of doing that to make alcoholic beverages and to make bread rise (via the carbon dioxide bubbles).

(d) DNA and protein production. Bacteria are great at making lots of copies of themselves, which involves making lots of copies of their DNA and their proteins. If you are a starving biology researcher and need to make lots of copies of a new gene or new protein as easily as possible, the simplest solution is to stick the gene into a harmless strain of *E. coli*, let them reproduce for a while, then extract the copies of the gene or the protein made by the gene from all of the little baby *E. coli*.

(e) Bacterial degradation and remediation involves finding natural bacteria or genetically engineering bacteria to eat, or at least to surround and hide, things we don't want in the environment, anything from oil spills to uranium ions. Now if only we could create bacteria that eat homework...

(f) Bacterial pesticides can be used instead of chemicals as a more environmentally benign method to kill certain insects. For example, *Bacillus thuringiensis* spores, bacteria, or their components (often sold as gardening supplies) can kill insect larvae before they develop into adult insects.

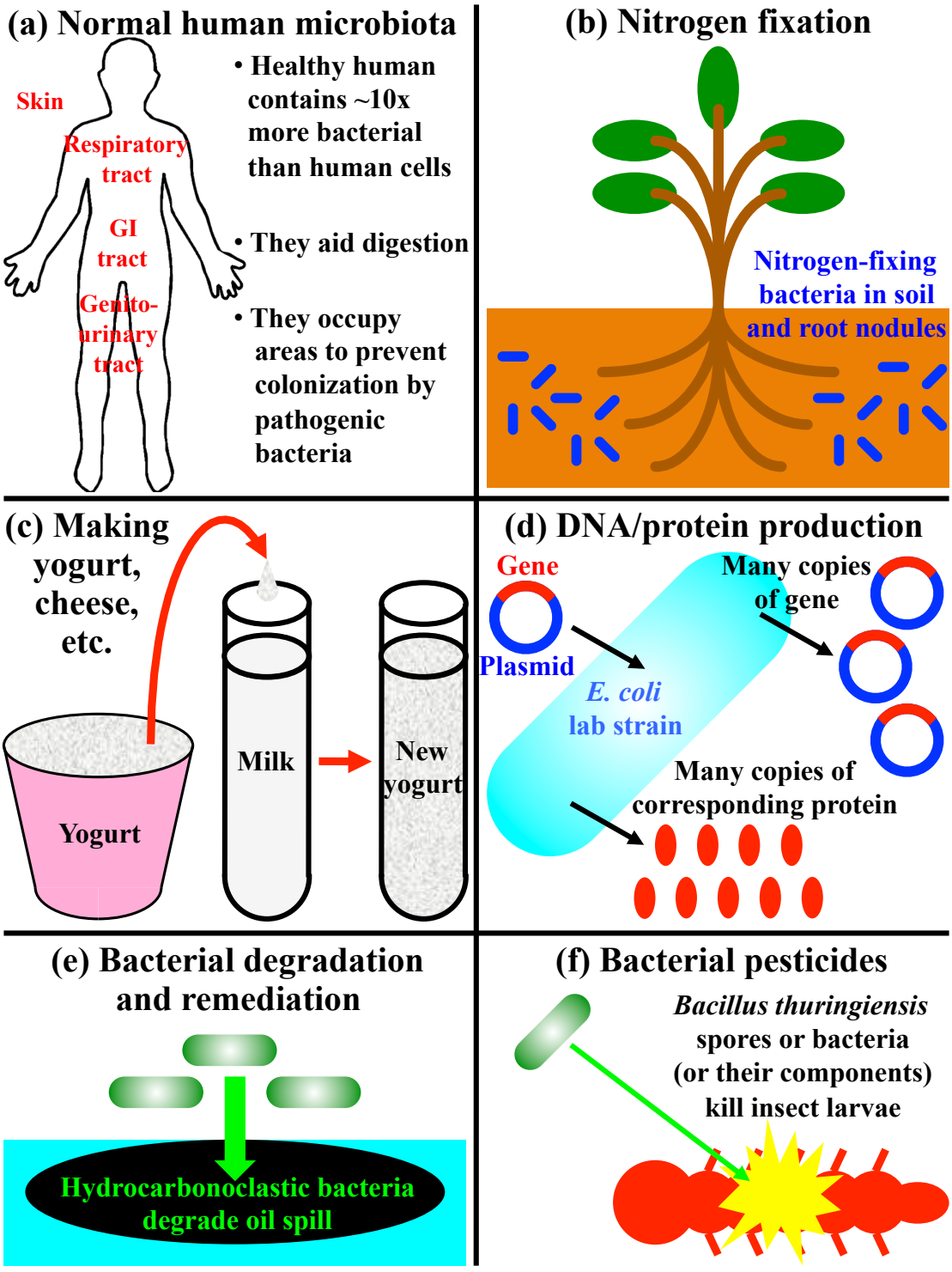


Fig. 34. Bacterial applications include: **(a)** normal human microbiota, **(b)** nitrogen fixation, **(c)** making yogurt, cheese, wine, etc., **(d)** DNA and protein production, **(e)** bacterial degradation and remediation, and **(f)** bacterial pesticides.

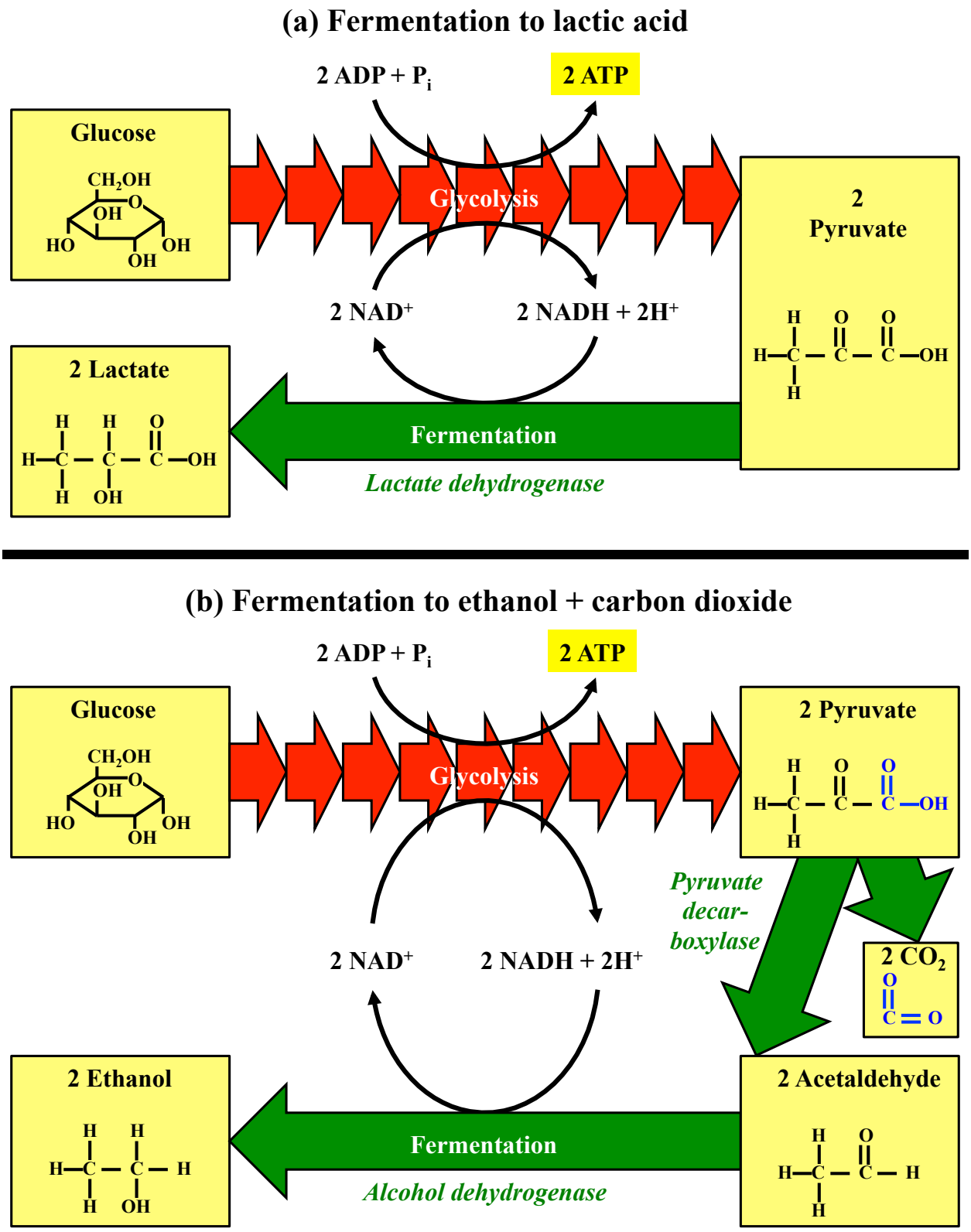


Fig. 35. Fermentation to (a) lactic acid and (b) ethanol plus carbon dioxide.

5 Fungi and Algae

Fungi and algae are eukaryotic (complex) cells similar to our own (Fig. 36). Fungal cells are surrounded by a thick cell wall composed of β-glucans, chitin, and mannoproteins, and fungi come in a wide variety, including those that make jocks itchy and hippies trippy. Algae are plant cells that are somewhat similar to fungi but with a different type of cell wall (usually composed of cellulose, glycan, and pectin), and with the ability to convert sunlight to energy via photosynthesis.

Although animal, plant, and fungal cells all have a lipid bilayer plasma membrane, the sterols within that membrane are different for animal cells (cholesterol), plant cells (phytosterol), and fungal cells (ergosterol), as illustrated in Figs. 36 and 37. As will be seen in Section 5.2, that comes in handy when you want to use drugs to kill one of them (generally fungi) but not the others.

There are a number of pathogenic fungi that will be covered below. Algae don't infect much except coral (which they actually help), since it is usually fairly dark inside an animal or human host. For more detailed information on algae, please see *Botany*.

5.1 Classification and Mechanisms of Fungi and Algae

As shown in Fig. 38, major categories of fungi include yeasts, molds, and mushrooms, although some fungi can behave like one category or another depending on the environmental conditions:

Fungi that appear as yeasts exist as single cells and reproduce by budding off to form a new cell, like Gremlins exposed to water. Some major pathogenic yeast species include:

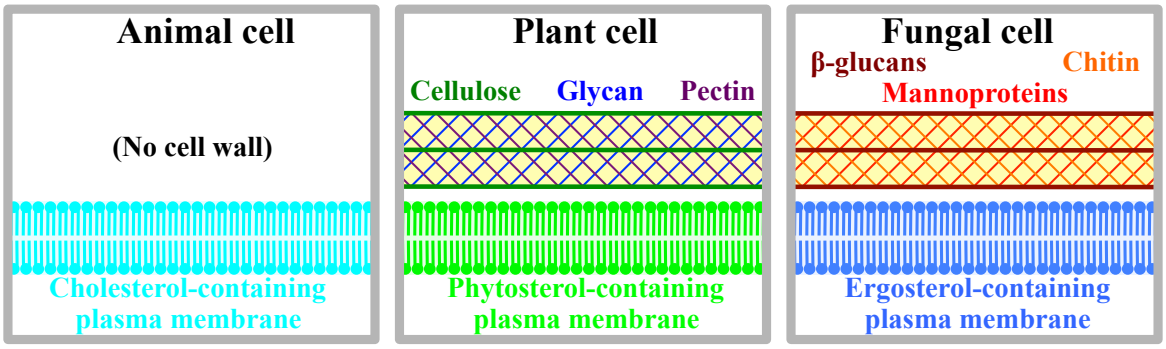
- *Coccidioides immitis*, *Histoplasma capsulatum*, and *Blastomyces dermatitidis* grow as molds at 25^{o}C in the environment and produce spores, which can be inhaled by humans. Inside a nice warm human, they convert to their yeast form, cause lung infections, and can potentially spread to other sites in the body (especially in patients with weakened immune systems).

- *Cryptococcus* species are honest to goodness yeast both inside and outside humans; like some bacteria, they are coated with a protective polysaccharide layer. If inhaled, they cause a mild lung infection and then head for the central nervous system, where they can cause a potentially fatal fungal mengingoencephalitis.

- *Candida albicans* is a yeast that is commonly found on human skin and if given the opportunity can cause vaginal infections, diaper rash on any skin areas that are presistently warm and moist, and oral infections (thrush). In immunocompromised patients, it can enter the body to cause infections inside various tissues.

Fungi that appear as molds form branched mycelium structures composed of lots of connected individual cells or hyphae. To reproduce, the ends of the branches form condidiophores that produce spores, which can remain dormant for long periods of time during harsh environmental conditions. If a spore finds a nice cozy spot, it germinates to become one hypha, divides into two hyphae, and opens a new franchise. Some favorite pathogenic mold species include:

- *Epidermophyton*, *Trichophyton*, and *Microsporum* cause fungal skin (tinea) infections, including nail infections, scalp infections, athlete's foot, jock itch, and ringworm on the body. They secrete a keritinase enzyme that breaks down the keratin structural protein in skin, nails, and hair, then live off the resulting nutrients.



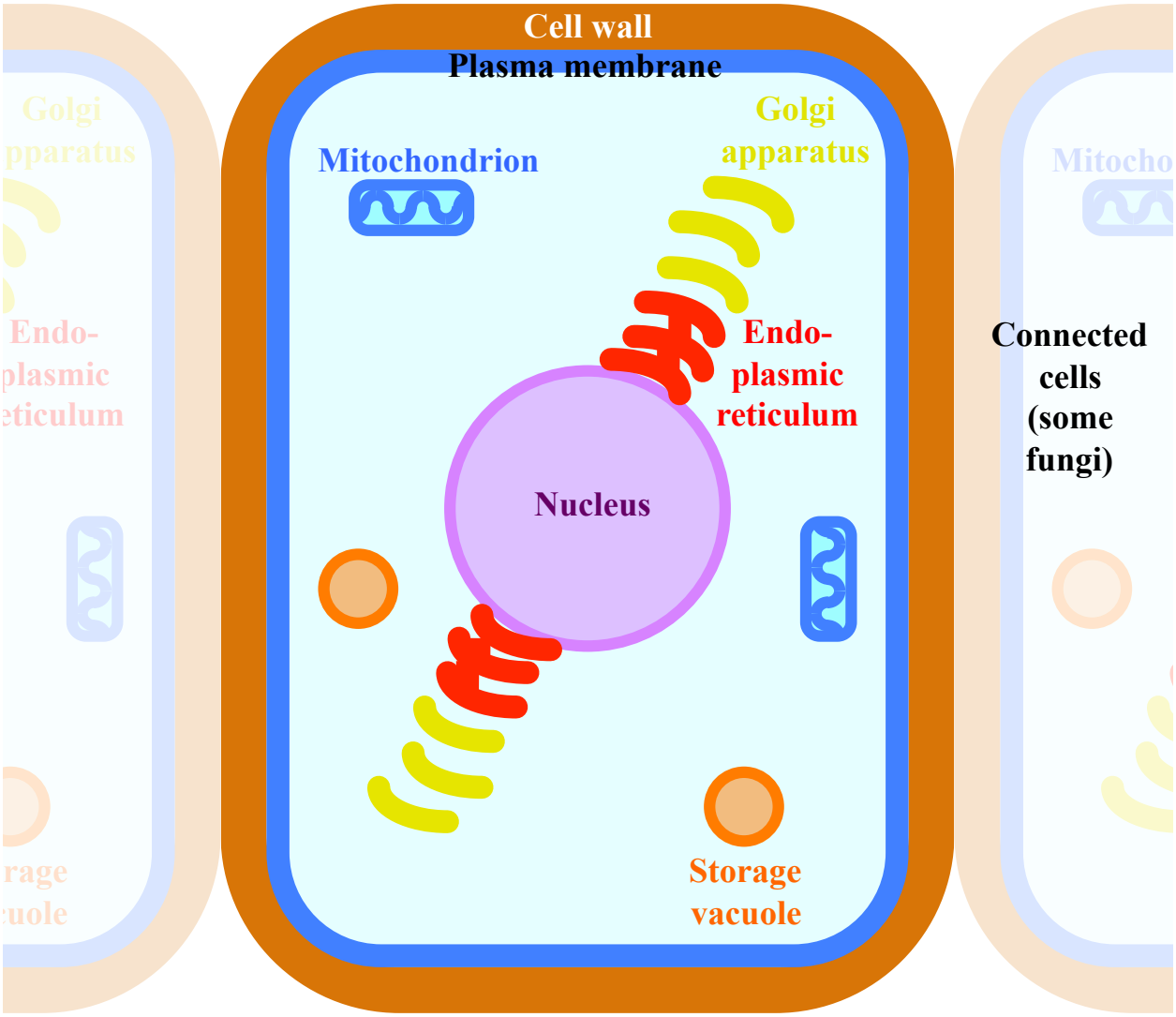


Fig. 36. Fungi. Animal, plant, and fungal cell membranes contain different types of sterols. Plant and fungal cells both have cell walls, but of different composition. Otherwise fungal cells look very similar to plant cells, but smaller and without chloroplasts.

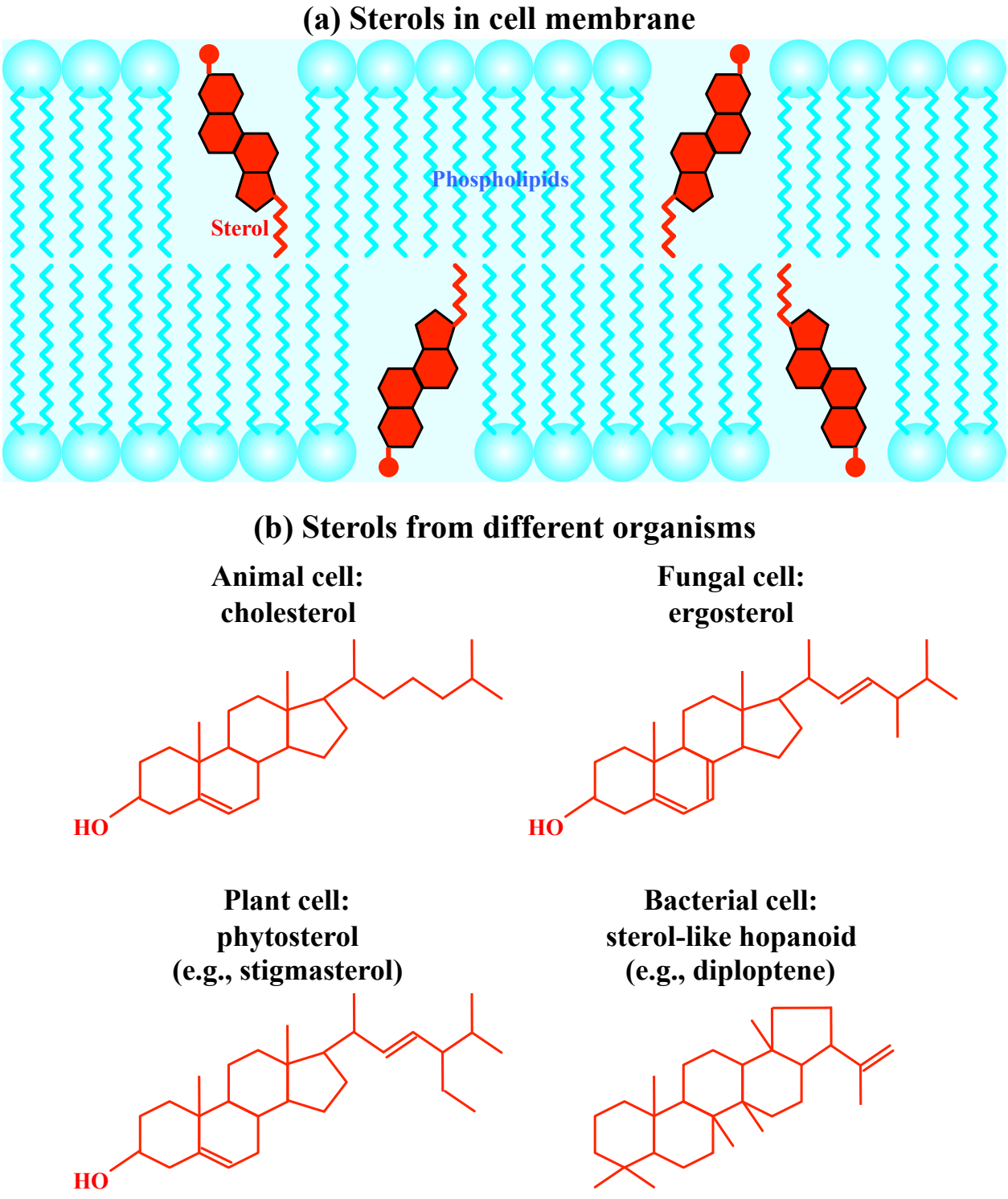


Fig. 37. Membrane sterols. **(a)** Location of sterols in cellular membranes. **(b)** Major types of sterols in membranes from different cell types. (In this compact and widely used notation, unless otherwise indicated, carbon atoms sit where lines end or change directions, and any of the four bonds of each carbon not otherwise used are occupied by hydrogens that are not shown).

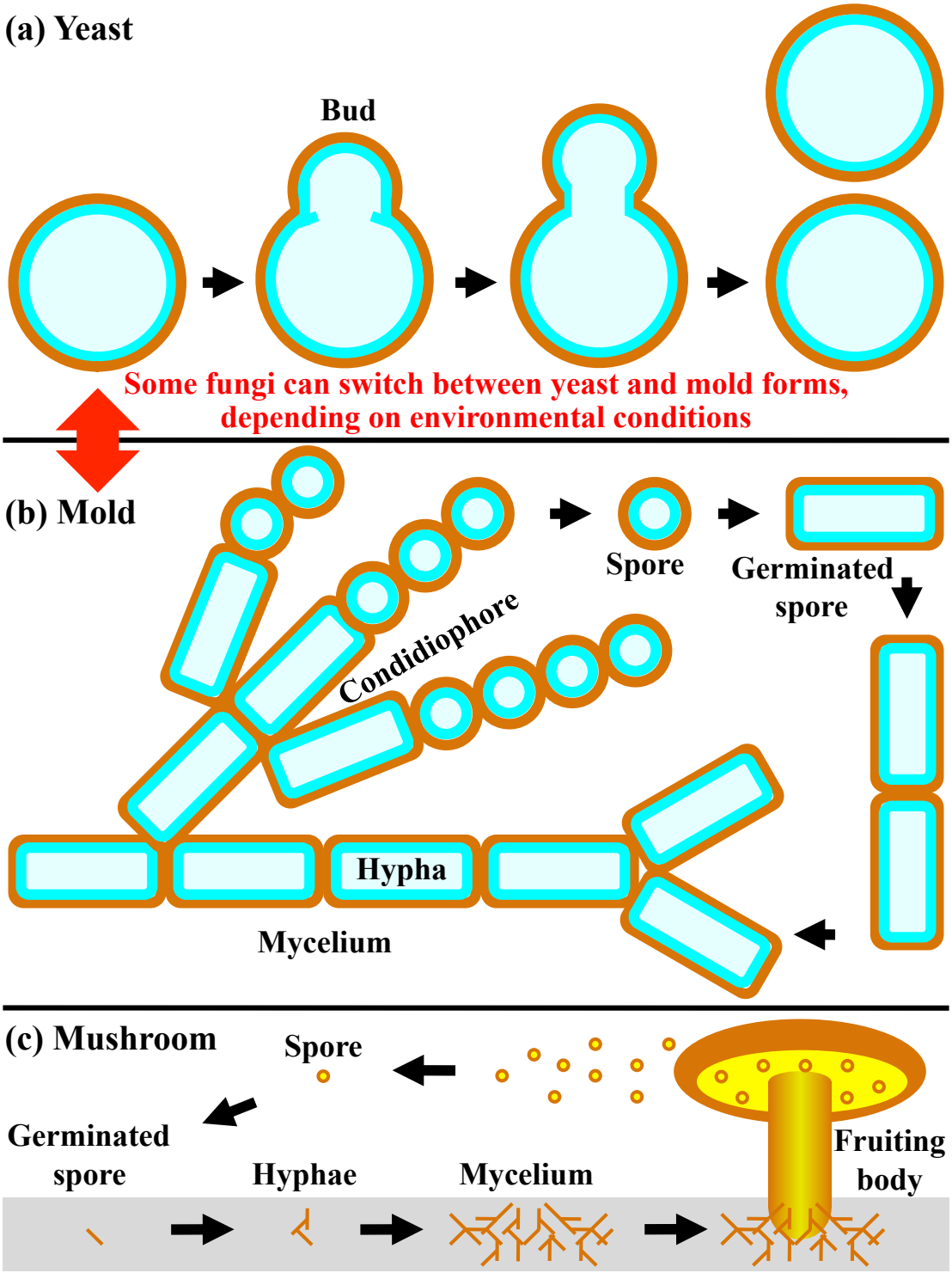


Fig. 38. Major categories of fungi include (a) yeasts, (b) molds, and (c) mushrooms, although some fungi can behave like one category or another depending on the environmental conditions.

- *Rhizopus*, *Rhizomucor*, and *Mucor* molds from the environment can cause potentially fatal sinus and/or lung infections in patients who are already seriously compromised by AIDS, diabetes, or other conditions.

- *Aspergillus* species are spore-forming molds widely found in the environment. If the spores are inhaled by a person, they can grow and cause respiratory infections, especially if the person is immunocompromised or has pre-existing respiratory problems. If the immune system is sufficiently weak, *Aspergillus* can potentially spread from the lungs to cause infections elsewhere in the body. Some *Aspergillus* species produce aflatoxin (which is both a toxin and a carcinogen) and can grow in stores of grains or nuts; the toxins can cause harm to people or animals that consume that food, even if the fungi themselves do not infect the people or animals.

Fungi that appear as mushrooms start off as simple spores and then hyphae and mycelia, but then they develop Tower-of-Babel-level ambitions and build a large fruiting body or mushroom from which to launch more spores. Here is why you should leave the mushrooms to the experts and not experiment:

- While many types of mushrooms are nontoxic and commonly used for food, some mushrooms produce toxins, presumably to dissuade animals from eating them in the wild. Different mushrooms produce different toxins, which have a wide variety of types and effects. Many toxins such as baeocystin, ergotamine, ibotenic acid, muscimol, psilocin, and psilocybin can cause temporary hallucinations or permanent neurotoxicity and may have non-neural effects as well (for example, ergotamine is a potent vasoconstrictor). A number of other toxins such as arabitol, bolesatine, and phallotoxin cause gastrointestinal problems including vomiting and/or diarrhea. Muscarine activates muscarinic acetylcholine receptors on muscles throughout the body, impairing everything from the heart to the lungs. Alpha-amanitin damages the liver and orellanine damages the kidneys.

Algae are simple plants that are often single-celled but can be multicellular. They are almost entirely free-living and nontoxic, with a couple of interesting exceptions:

- **Symbiotic algae** can live beneficially with or inside another organism, using their capacity for photosynthesis to provide that organism with nutrients if the algae are close enough to the surface to still receive sunlight. Major examples include lichens (a mixture of algae cells and fungal cells), algae growing near the surface inside coral reefs, and algae growing near the surface inside green ocean sponges.

- **Harmful algal blooms** can result from overproduction of algae in the ocean. Even in the best case, an algal bloom can suck a lot of nutrients out of the water and thereby deprive other species that need those nutrients. In the worst case, some species of dinoflagellate algae produce toxins that are harmful in the environment, and even more harmful if they accumulate in local sea critters that are then eaten by humans.

5.2 Antifungal Therapeutics

While fungal cells are relatively similar to human cells, antifungal therapeutics target some of the key features that distinguish fungal cells from human cells:

(a) Cell wall inhibitors target the unique components in fungal cell walls:

- Currently the main fungal cell wall inhibitors are echinocandins including caspofungin, micafungin, and anidulafungin.

(b) Microtubule inhibitors interfere with the cytoskeletal rearrangement necessary for yeast cells to bud to produce new cells:

- Griseofulvin is the major current example.

(c) Nucleoside analogues can potentially target fungal cells by being absorbed and incorporated more in fungal cell DNA/RNA than in human cell DNA/RNA:

- Currently the main example for antifungal applications is flucytosine.

(d) Ergosterol inhibitors exploit the fact that fungal plasma membranes contain ergosterol, whereas human plasma membranes contain cholesterol (Fig. 37):

- Polyenes such as amphotericin B, nystatin, natamycin, and candicidin directly attack ergosterol molecules, punching holes in the plasma membranes of fungal cells.

- Terbinafine and lots of "-zoles" (clotrimazole, fluconazole, ketoconazole, miconazole, posaconazole, traconazole, voriconazole, etc.) inhibit fungal enzymes involved in synthesizing ergosterol.

Since human cells have microtubules, DNA/RNA, and cholesterol pathways (if not ergosterol pathways), all of the above drugs except the cell wall inhibitors can have side effects or outright toxicity in humans. That is the difficulty of fighting bad guys that look so similar to the good guys.

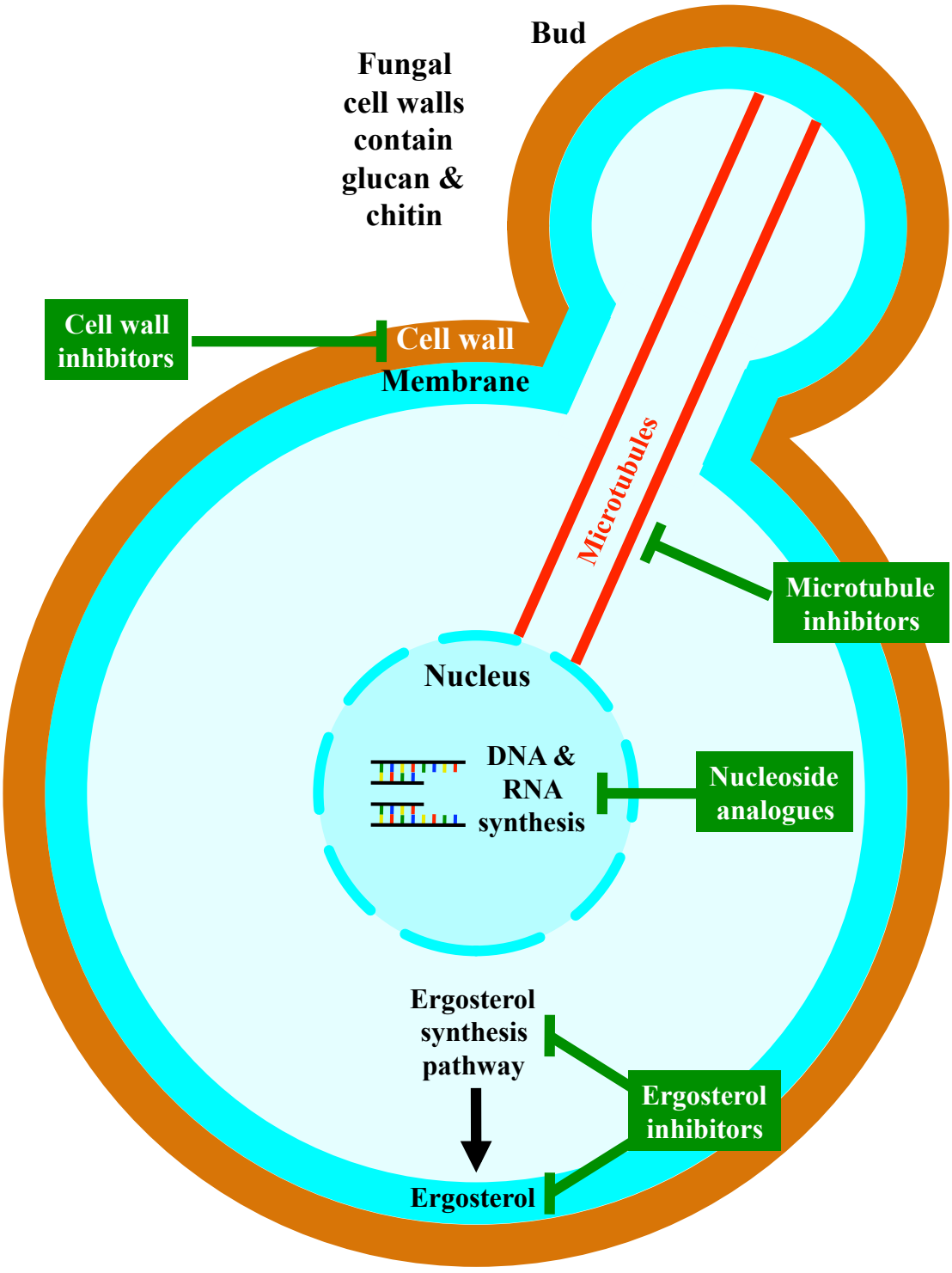


Fig. 39. Major categories of therapeutics for fungal infections include cell wall inhibitors, microtubule inhibitors, nucleoside analogues, and ergosterol inhibitors.

5.3 Useful Applications of Fungi and Algae

Many fungi and virtually all algae make themselves useful, both in the environment and in the lab:

- Yeast, especially the species *Saccharomyces cerevisiae* (*S. cerevisiae*), have been used as model organisms in the lab to study how eukaryotic cells work. Many of the genes, proteins, and pathways that have been discovered this way are also relevant to more complex eukaryotic cells like human cells.

- Since yeast are eukaryotic cells yet relatively simple and hardy ones, they have been used in the lab for protein production after new genes encoding proteins of interest are inserted into the yeast. Some of the species most widely used for this purpose are *S. cerevisiae*, *Pichia pastoris*, and *Hansenula polymorpha*.

- *S. cerevisiae* and other yeast species are used for fermentation during food production. Via the pathway in Fig. 35(b), yeast can convert sugar to ethanol (ethyl alcohol) plus carbon dioxide (CO_2) gas. That is used to convert different types of sugary liquids (juices, etc.) into different alcoholic beverages ranging from beer to wine. The CO_2 production is also used to create gas bubbles that make bread rise (while the resulting alcohol is boiled off during baking).

- Fungi and algae play vital roles in the environment and in food chains, absorbing nutrients from other things, and making or becoming nutrients for lots of other critters.

- One biotechnology holy grail is to genetically engineer algae cells that can convert sunlight into high-quality fuel. Lots of mad scientists have tried; the results are promising but thus far not economically viable or sufficiently practical to implement. Maybe someday they will succeed. Or maybe the experiments will go awry and the world will be taken over by *The Green Slime...*

- Algae all over the planet are nice enough to produce oxygen for all the rest of us to breathe. That was the source of the first atmospheric oxygen (from cyanobacteria, photosynthetic algae-like bacteria) and remains a major source, since land plants couldn't make enough just by themselves. Be grateful to pond scum every time you breathe.

6 Protozoa

Protozoa or **protists** are single-celled animals, so they are eukaryotic cells that very similar to our own cells. Meaner ones can take up residence in humans and cause disease either from inside or from outside our cells, whereas nicer ones just float around harmlessly in the environment. Most protozoa can form spore-like cysts that are ∼10-20 μm wide and can lie dormant during harsh conditions in the environment, then in better conditions can hatch and grow to their mature forms, which are usually ∼20-400 μm long.

6.1 Classification and Mechanisms of Protozoa

Generations of biologists have fought and killed each other over the best way to classify protozoa, and there is still no clear resolution. Here we will go with the simplest system to classify protozoa, based on how they move around. Different protozoa use four main categories of locomotion:

(a) Ciliates use cilia, like short waving hairs or oars on a boat, to paddle around. Figure 40 shows some major types of ciliates:

- *Paramecium* is a generally harmless and very widespread genus of protozoa found in unpurified water and biology classrooms worldwide. Paramecia are noteworthy for having their genome divided between a larger macronucleus and a smaller micronucleus.
- *Balantidium coli* is the evil sibling of paramecia. It also has a macronucleus and a micronucleus, but its cysts can be ingested by humans or animals, grow up in the intestine, and cause diarrhea.

(b) Flagellates use flagella, a whip-like tail on each cell (or sometimes a few tails per cell) to swim around. Some noteworthy flagellates are illustrated in Fig. 40:

- *Euglena* are not pathogenic but they are really cool anyway. They are swimming protozoa like single-celled animals, yet they have photosynthetic chloroplasts like plants. They or similar swimming algae-like critters are widely found in environmental water that is exposed to daily sunlight.
- *Trypanosoma* has several species, the most important of which are *Trypanosoma cruzi* (which is spread by South American triatomine bugs and causes Chagas disease) and *Trypanosoma brucei* (which is spread by African tsetse flies and causes sleeping sickness). As shown in Fig. 41, trypanosomes are spread by bug bites, replicate inside skin cells near the site of initial infection, burst out of those cells and travel through the bloodstream, and enter and infect cells elsewhere in the body. The trypanosomes can cause infections in almost any tissues, but *T. cruzi* is especially fond of damaging the heart, and *T. brucei* is especially fond of damaging the brain.
- *Leishmania* flagellates are very similar to *Trypanosoma* except they hitch rides on sandflies. Like trypanosomes, Leishmania protozoa start as a skin infection at the bite site, then go on a concert tour. They can go almost anywhere but are as fond of damaging the spleen and liver as rock bands are of damaging hotel rooms.
- *Giardia* cysts that are ingested can grow into mature protozoa, cause gastrointestinal illnesses, and produce cysts that are excreted to infect other folks, as illustrated in Fig. 42. They interfere with fat absorption in the body more than other pathogenic intestinal protozoa do, making the resulting diarrhea especially greasy and nasty smelling. Now try forgetting that...
- *Trichomonas* causes sexually transmitted infections of the urogenital tract and is anaerobic, since you really wouldn't want to breathe down there.

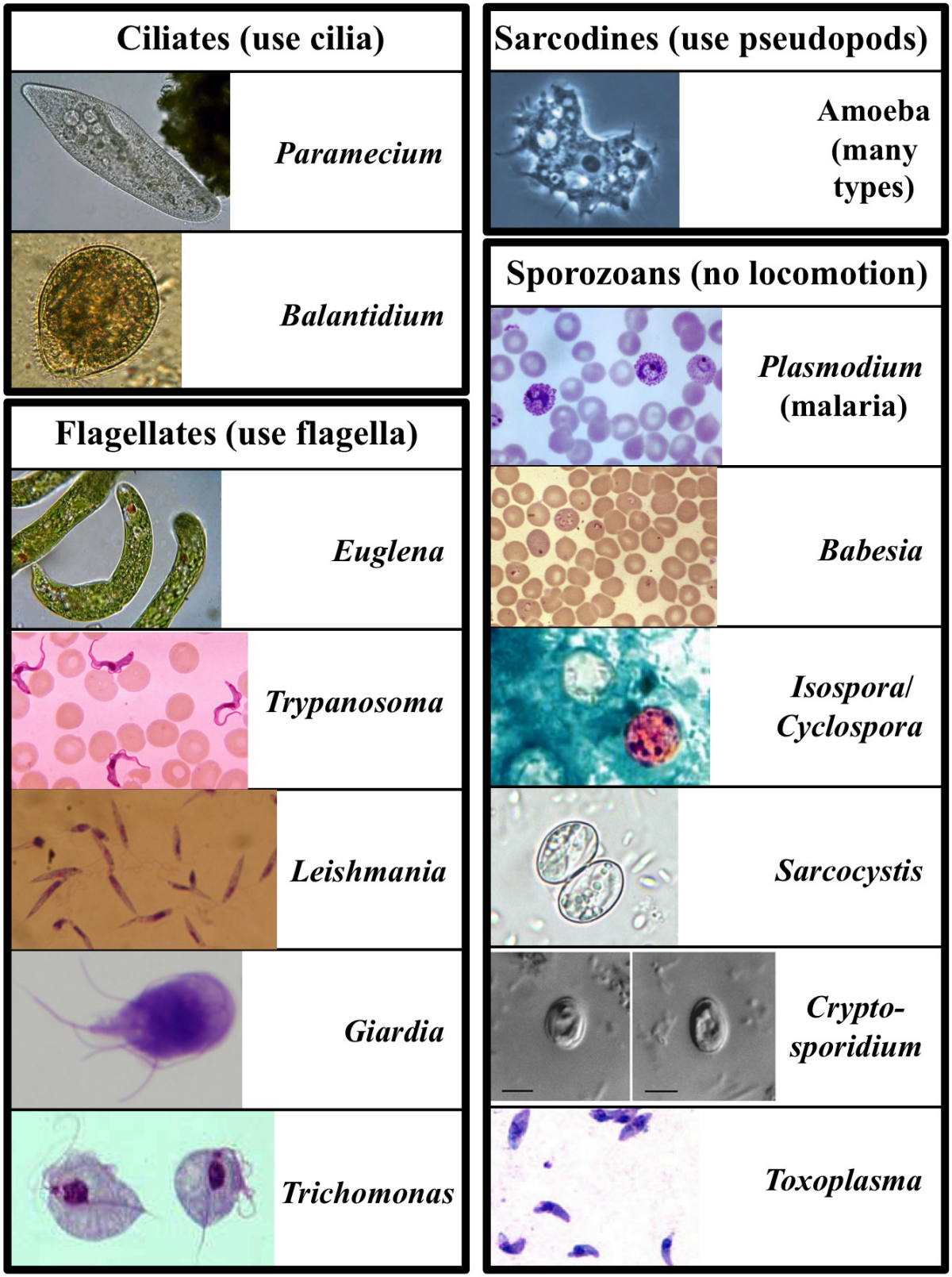


Fig. 40. Major categories of protozoa based on their method of locomotion, including ciliates, flagellates, sarcodines, and sporozoans.

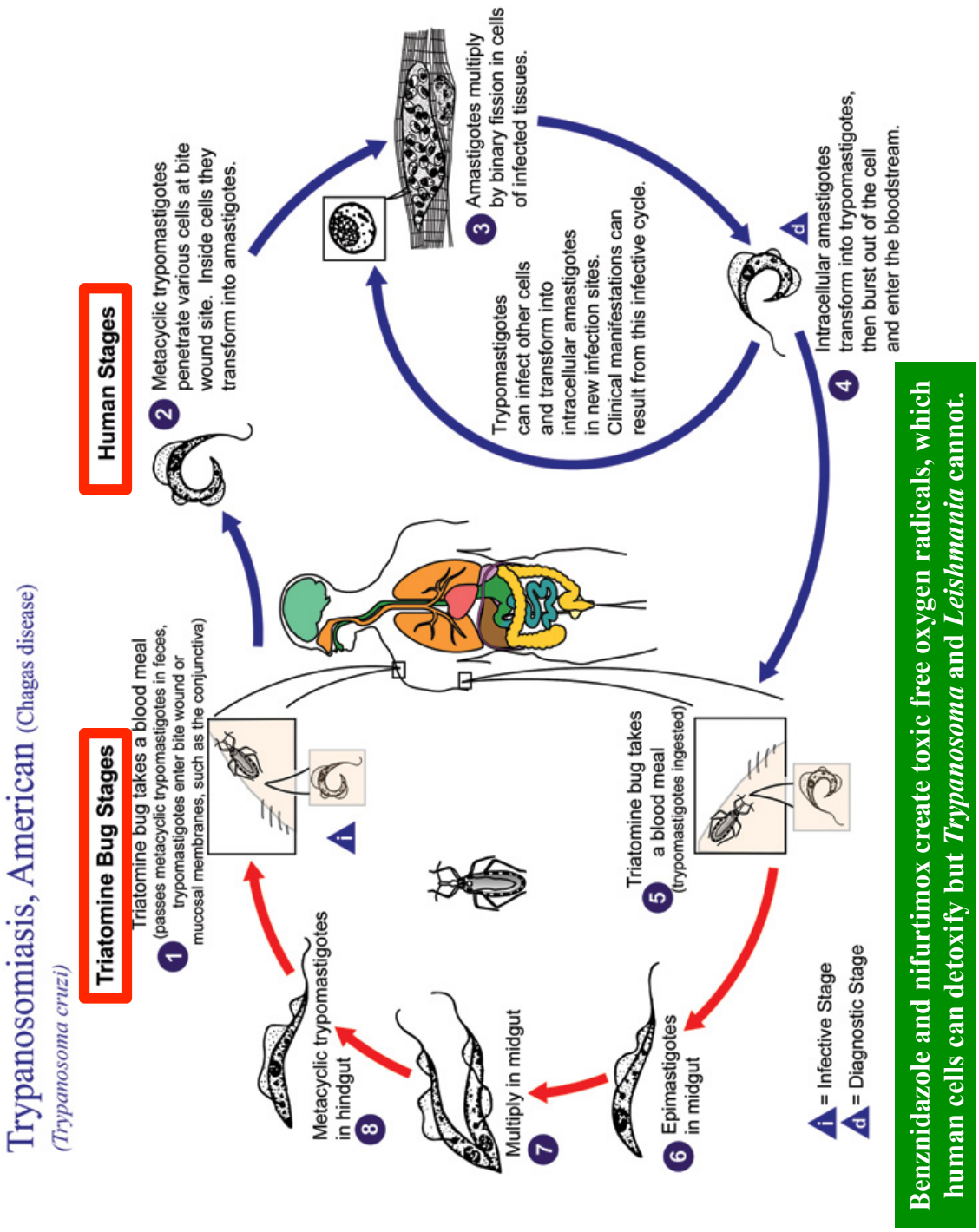


Fig. 41. ***Trypanosoma*** **replication cycle and therapeutics** (adapted from public domain-image from U. S. Centers for Disease Control).

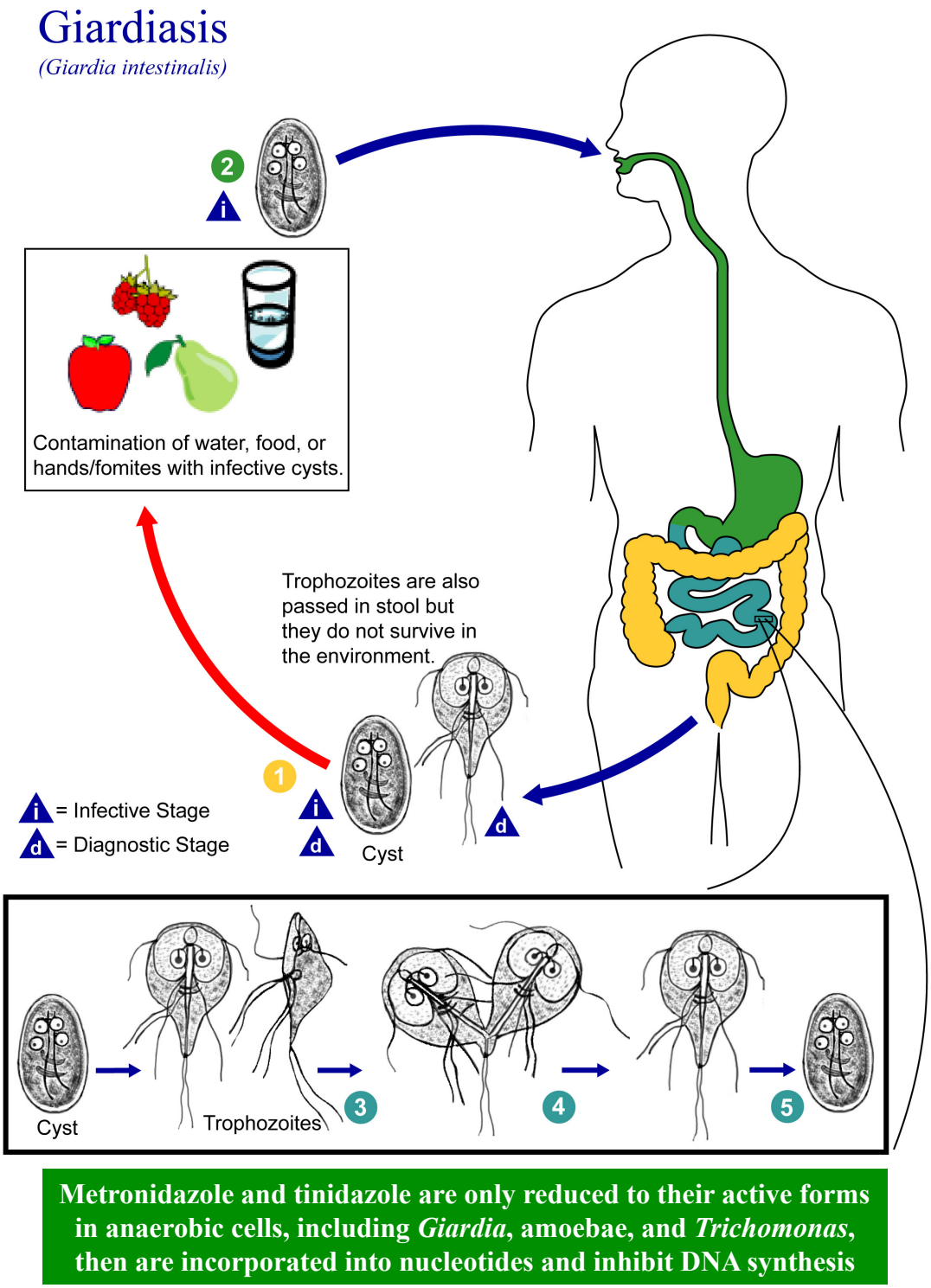


Fig. 42. ***Giardia*** **replication cycle and therapeutics** (adapted from public domain-image from U. S. Centers for Disease Control).

(c) Sarcodines are amorphous blobs that move around using pseudopods, temporary leg-like protrusions that can form and then dissipate on any side of the cell where they are needed. It is just too darned difficult to tell one amorphous blob from another (Fig. 40), so they are all called:

- Amoeba. Some amoebae happily live in the environment and do not cause disease, some can infect humans or animals and cause disease (mainly gastrointestinal illness [e.g., *Entamoeba histolytica*], with your occasional brain-eating amoeba [e.g., *Naegleria fowleri*] thrown in for dramatic effect), and some can go either way depending on their opportunities in life.

(d) Sporozoans are lazy bums that have no method of locomotion of their own, so they just float around whichever way the surrounding fluid or surfaces carry them. Figure 40 depicts some famous sporozoans:

- *Plasmodium* sporozoans cause malaria. As shown in Fig. 43, they fly first class in mosquitoes, which bite humans and transfer the sporozoans to the bloodstream. They travel to the liver, where they infect, grow, and replicate inside hepatocytes (liver cells), bursting out of the cells once they are done. While some stay and attack other hepatocytes, most then migrate into red blood cells, which they eat from the inside out like the world's tiniest vampires. The herd of *Plasmodium* sporozoans go through synchronized rounds where they all go into new red blood cells and munch inside them for a while, and then they all burst out of those cells and repeat the cycle with more red blood cells. The body's immune system reacts with a strong inflammatory response and fever each time it sees the sporozoans outside the red blood cells, but not really when they are hiding inside the red blood cells. Thus malaria has waves of fever that come and go with regular frequency. Different species of *Plasmodium* have different frequencies, ranging from 36-48 hours per round of replication for *P. falciparum* to 48 hours for *P. vivax* to 72 hours for *P. malariae*.

- *Babesia* sporozoans are a heck of a lot like *Plasmodium* and also munch on your red blood cells from the inside out, except when traveling they prefer to book passage on *Ixodes* ticks instead of mosquitoes, presumably since they get better customer reward points.

- *Isospora* and *Cyclospora* cysts that are ingested with food or water can grow into mature protozoa, cause gastrointestinal illnesses, and produce cysts that are excreted. After a few weeks in the environment, those cysts convert to an infectious form that can torment somebody else if they are ingested.

- *Sarcocystis* can form cysts in the muscle of infected animals, then cause gastrointestinal illnesses if humans consume that meat without sufficiently cooking it.

- *Cryptosporidium* cysts that are ingested can grow into mature protozoa, cause gastrointestinal illnesses, and produce cysts that are excreted, just like *Cyclospora*. Unlike *Cyclospora*, *Cryptosporidium* cysts are immediately infectious without having to develop in the environment, and can also cause respiratory infections if they are coughed up from the intestine to the throat and then inhaled into the lungs.

- *Toxoplasma gondii* likes to live inside cells, especially in cats. If it infects rodents, it makes them more likely to get caught by cats. If if infects humans, it makes them more likely to buy more cats. Other than possibly turning into a crazy cat person, most humans who are exposed develop a very low-level, asymptomatic, lifelong infection. However, if a woman is exposed to *Toxoplasma gondii* for the first time while she is pregnant, the fetus may be stillborn or have retinal and/or neural birth defects.

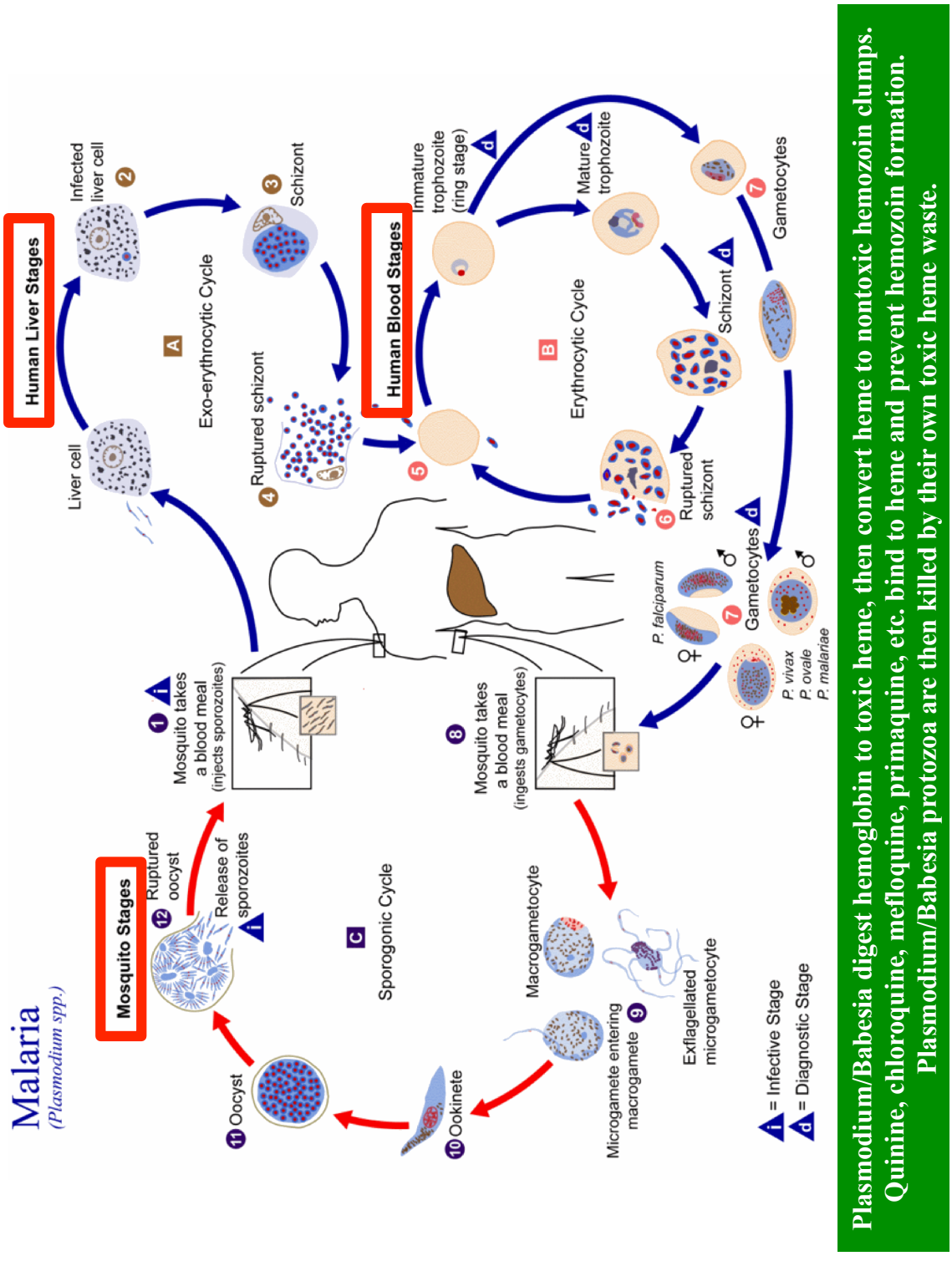


Fig. 43. ***Plasmodium*** **or malaria replication cycle and therapeutics** (adapted from public domain-image from U. S. Centers for Disease Control).

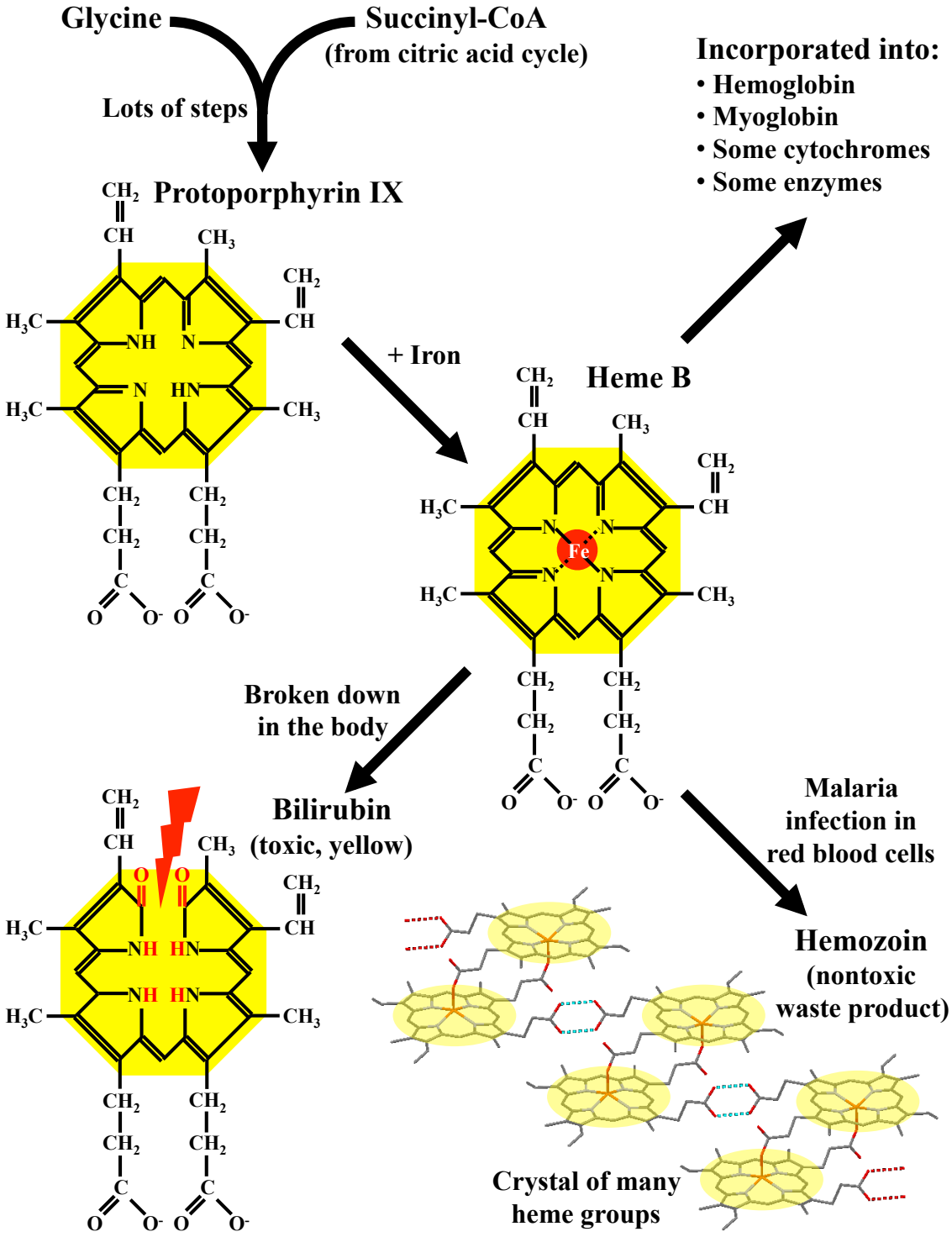


Fig. 44. Synthesis and degradation of heme.

6.2 Anti-Protozoan Therapeutics

Protozoa are individual animal cells and humans are composed of animal cells, which means that most drugs that might kill the protozoa would also kill human cells. The trick is to find some feature that is significantly different between the protozoa and human cells. Some of the major strategies behind anti-protozoan therapeutic drugs include:

- Benznidazole and nifurtimox create toxic free oxygen radicals, which human cells can detoxify (using natural human enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase) but *Trypanosoma* and *Leishmania* protozoa cannot. Thus when these drugs are taken up by the protozoa, they create free oxygen radicals, which in turn produce enough oxidative damage to their membranes, proteins, DNA, and other cellular components to kill the protozoa.

- Metronidazole and tinidazole are only reduced to their active forms in anaerobic cells that live without oxygen, including *Giardia*, amoebae, and *Trichomonas*. The reduced/activated forms of these drugs are then incorporated into nucleotides and inhibit DNA synthesis and replication of the protozoa. Human cells are aerobic, consuming oxygen supplied from the bloodstream, so they do not reduce metronidazole and tinidazole to their active forms and thus are not harmed by them.

- *Plasmodium* and *Babesia* protozoa eat your red blood cells from the inside out by digesting hemoglobin to toxic heme waste products and gaining some energy in the process. To avoid poisoning themselves with their own toxic heme waste, the protozoa convert toxic individual heme molecules into nontoxic hemozoin crystals or clumps that precipate out of solution, as shown in Fig. 44. Most of the major drugs for these infections (quinine, chloroquine, mefloquine, primaquine, etc.) bind to individual heme molecules and prevent formation of hemozoin clumps. Thus the *Plasmodium* or *Babesia* protozoa end up getting killed by their own toxic heme waste that they can no longer eliminate. Artemisinin compounds are somewhat different, in that they are activated by excessive heme in protozoa-infected red blood cells and produce free oxygen radicals that damage and kill the protozoa.

6.3 Useful Applications of Protozoa

Although some protozoa are pathogenic and like to live in a human or animal host, most protozoa live on their own in the environment and perform beneficial functions. Those with chloroplasts like *Euglena* can photosynthesize to produce oxygen and nutrients for everybody else. Many protozoa in the water and soil eat and help to break down waste products from larger organisms or remains of dead organisms. The protozoa also form a link in the food chain, eating anything smaller than themselves (bacteria, fungi, and nutrients from the environment) and in turn being eaten by larger animals. Most importantly, protozoa in pond water have entertained generations of biology students with microscopes and provided gainful employment for countless biology teachers who would otherwise have lived lives of abject poverty.

7 Helminths

And now for your reading pleasure, we have saved the grossest topic for last. "Helminth" is biologyese for "worm." How did worms end up in a microbiology survey? Whereas most worms stay in the ground where they belong, some fortune-seeking worms can enter the body of a human or an animal host when the worm is still at a microscopic stage of its development. Then they live off the nutrients provided by the host's body, growing to be quite large and thoroughly disgusting within the body of their host. It is estimated that at least 2-3 billion people worldwide are infected with one or more types of helminths.

7.1 Classification and Mechanisms of Helminths

As shown in Fig. 45, helminths may be divided into three broad categories based on their shapes. Most helminths generally follow the life cycle depicted in Fig. 46, although exceptions will be noted below:

(a) Nematodes or roundworms are basically tubes, where nutrients go in the mouth end and waste goes out the anus end, as illustrated in Fig. 45(a). They usually have female and male genders to produce eggs and sperm, but sometimes they have sexual identity issues. Some major disease-causing nematodes are:

- *Ascaris* nematodes follow the life cycle shown in Fig. 46. Microscopic larvae in the environment are accidentally ingested by humans (or animals), get swallowed to the intestine, penetrate into the bloodstream and take a field trip to the lungs, get coughed up to the esophagus and swallowed back to the intestine, and finally decide to settle down, get married, and buy some real estate. Fertilized eggs are excreted in feces, hatch into larvae in the environment, and infect somebody else. *Ascaris* worms can grow up to 35 cm long, and in severe cases herds of worms can completely obstruct the intestine, especially in younger children.

- *Trichuris* whipworms mostly do the same thing, except mature worms are smaller ($\sim$5 cm long), and once swallowed, the critters just stay in the intestine without taking a sightseeing trip through the lungs.

- *Enterobius* pinworms are much smaller, $\sim$ 1 cm long or less when mature. Once swallowed they also stay in the intestine. One uniquely gross feature is that the female temporarily emerges from the host's anus to deposit fertilized eggs on the surrounding skin, where they cause rectal itching and can be accidentally picked up by scratching fingers.

- *Ancylostoma* and *Necator* hookworms are also $\sim$ 1 cm long when mature. Their larvae penetrate from the soil directly through the skin into a human host, travel through the bloodstream to the lungs, yada-yada, get coughed up and swallowed to the intestine, mature and mate there, and release fertilized eggs in feces, which hatch into infectious larvae in the environment.

- *Trichinella* is the smallest human nematode and the largest intracellular parasite. *Trichinella* larvae are ingested, penetrate the intestinal wall, and set up shop *inside* individual muscle cells either in the intestinal wall or elsewhere in the body. Humans can become infected by eating undercooked animal muscle containing larval cysts. Larvae mature to adults in the intestine and release fertilized eggs in feces.

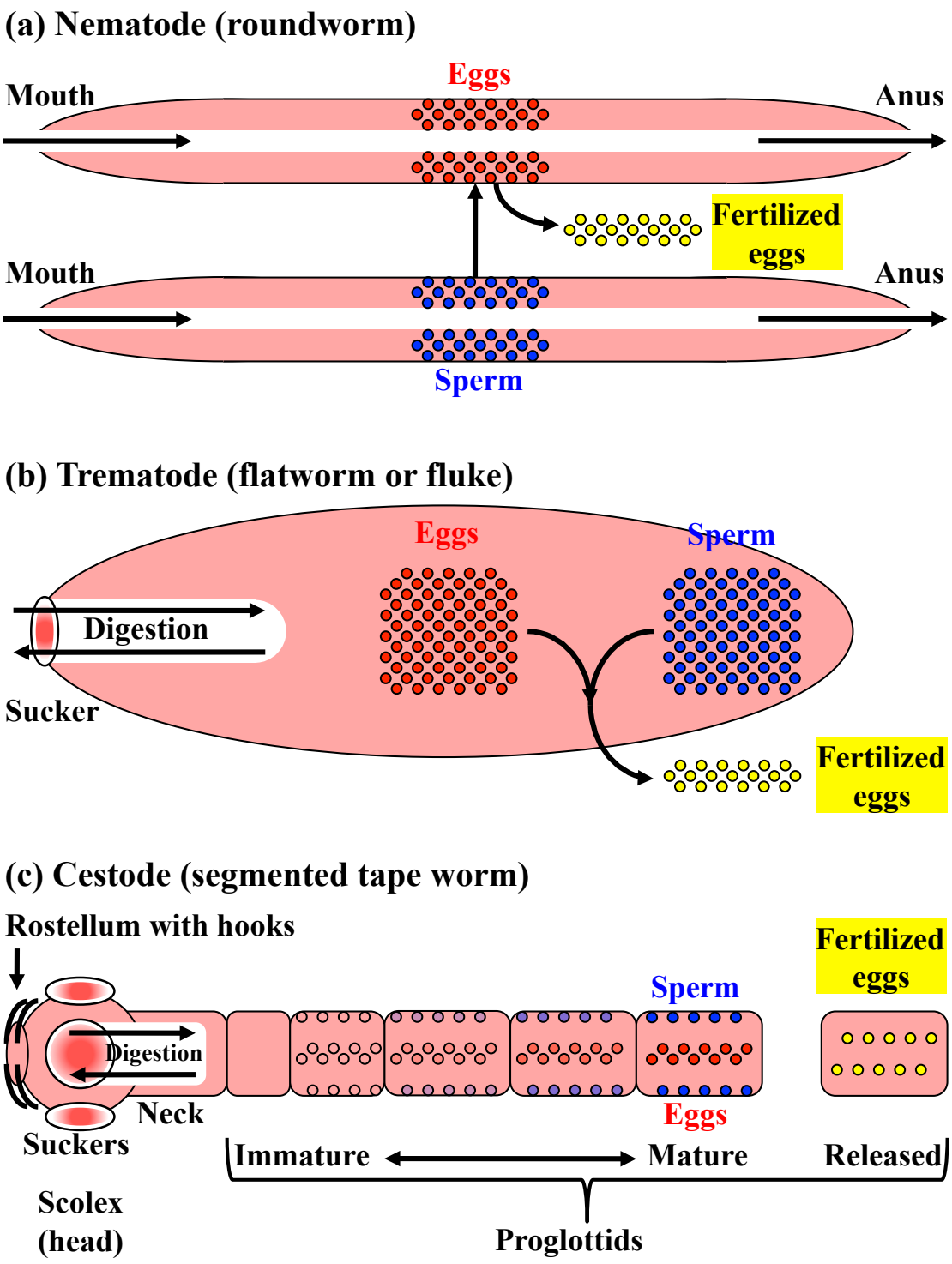


Fig. 45. Helminths include: **(a)** nematodes or roundworms; **(b)** trematodes, flatworms, or flukes; and **(c)** cestodes or segmented tape worms.

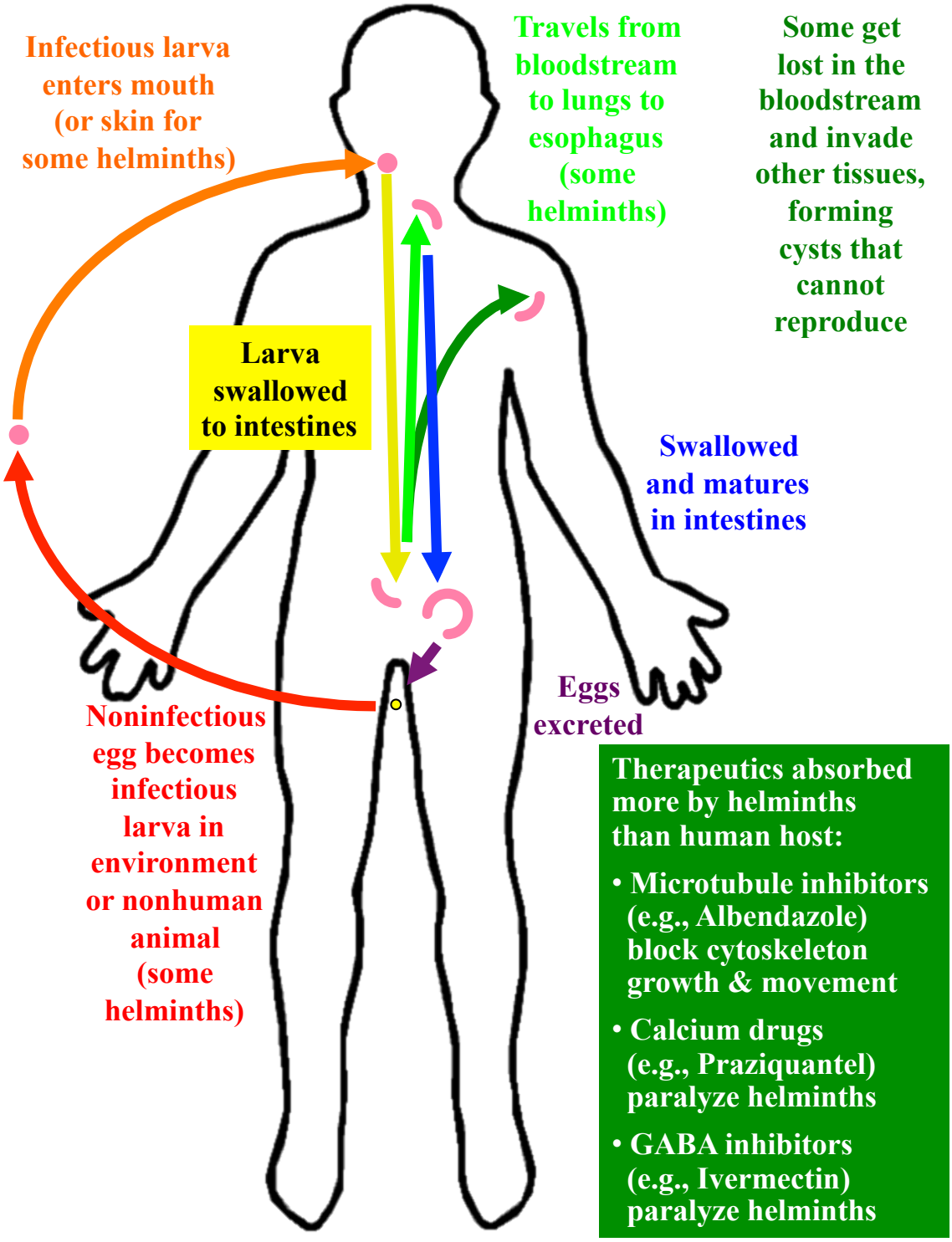


Fig. 46. Common life cycles and therapeutics for pathogenic helminths.

- *Dracunculus* or Guinea worm larvae are eaten by microscopic crustaceans, which may be ingested by humans drinking unfiltered water. In humans, the male and female larvae mature in the intestine, and female nematodes with fertilized eggs migrate to the skin. Part of the female slowly emerges from a blister, shedding larvae into the environment. To remove the worm without breaking it and releasing worm goo that would cause anaphylactic shock and death of the human host, the protruding part of the worm must be slowly wound around a stick by a few centimeters a day for several weeks. How would you like that hobby?

- *Onchocerca* or African river blindless larvae are ingested by black flies biting infected humans, mature somewhat inside the flies, and are transmitted to new humans that the flies bite. In humans, the larvae mature into male and female nematodes and mate to produce fertilized eggs, which hatch into larvae that migrate to the skin to create itchy bumps and attract black flies. Larvae may also migrate through the bloodstream and end up swimming around inside the eyes, causing blindness and looking really freaky to anyone who peers into your eyes.

- *Wuchereria* and *Brugia* elephantiasis larvae are ingested by mosquitoes biting infected people (or animals), mature somewhat inside the mosquitoes, and are transmitted to new people that the mosquitoes feed on. In humans, the larvae migrate into the lymphatic system, where they mature, mate, and release larvae into the bloodstream, to be taken up by more mosquitoes. Blockage of the lymphatic system by adult worms, and inflammatory responses to the worms, cause grotesque lumpy swelling or elephantiasis of the affected parts of the host's body.

- *Dirofilaria* or heartworm larvae are ingested by mosquitoes biting infected dogs (or occasionally cats or other animals), mature somewhat inside the mosquitoes, and are transmitted to new dogs that the mosquitoes feed on. In dogs, the larvae migrate into muscles, grow there a while, then migrate to major arteries in the heart and lungs, where they mature, mate, and release larvae into the bloodstream, to be taken up by more mosquitoes. If there are enough worms or if the dog is fairly small, pulmonary and/or cardiovascular function can be significantly impaired.

(b) Trematodes, flatworms, or flukes tend to be wider and flatter than nematodes, as illustrated in Fig. 45(b). They attach to a preferred target organ inside their host via a sucker, which they use both to ingest nutrients and to expel waste. Also in the interest of simplicity, most trematodes are sweet transvestites from transexual Transylvania, producing both eggs and sperm that are combined to create fertilized eggs. Depending on the species, mature trematodes can grow to be 1-10 cm long. They can produce significant damage or physical obstruction in their target organ, cause systemic anaemia or malnutrition, and provoke an inflammatory response that does more damage to the host's tissues than to the trematode. Some major trematodes include:

- Lung flukes (*Paragonimus*) are very patient; they begin as fertilized eggs that hatch in fresh water and infect snails, which get eaten by crabs or crayfish, which get eaten by humans. Once eaten by a human, larvae migrate from the intestine to the lungs, where they mature and produce fertilized eggs. Fertilized eggs are coughed up, swallowed, and excreted in feces to complete the lung fluke circle of life, except it isn't as warm and fuzzy as *The Lion King*.

- Some liver flukes (*Clonorchis* and *Opisthorchis*) begin in fresh water as fertilized eggs that infect snails, emerge as microscopic larvae that penetrate a fish's skin, and set up temporary residence in the fish's muscles. If the fish is eaten by a human, the larvae migrate from the intestine to the liver, where they attach themselves, feed on bile, and mature to produce fertilized eggs that are excreted in feces. Other liver fluke larvae (*Fasciola*) follow the same itinerary except they hitchhike on a water plant instead of a fish.

- Blood flukes (*Schistosoma*) begin as fertilized eggs that hatch in fresh water, infect snails, emerge as microscopic larvae that can penetrate directly through human skin, migrate to capillaries near the bladder or intestine, and mature there. Unlike other trematodes, they do have females and males, but they physically join together for the rest of their lives, so in practice maybe there isn't such a vas deferens after all. They release fertilized eggs in the urine and/or stool, completing the cycle.

(c) Cestodes or segmented tape worms have a head or scolex followed by a bunch of segments or proglottids. The head has hooks that attach to the intestinal wall in a host, and suckers that ingest nutrients and expel waste. The segments produce both eggs and sperm, with the newest segments after the head being immature and still developing, and the oldest segments further from the head mature enough to produce fertilized eggs. Very mature segments can break off to release their cargo of fertilized eggs in feces, from which they can infect new hosts. Different species of cestodes live in different natural hosts and may be acquired by humans by different routes. Cestodes start off as microscopic fertilized eggs but grow to various sizes, depending on the natural host they are used to inhabiting. Beef tapeworms can grow to be up to 20 meters long. Some famous cestodes include:

- *Taenia* eggs hatch in pigs or cattle, migrate from the intestine through the bloodstream to muscle, encapsulate themselves in protective cysts, and wait patiently. If a human eats that pork or beef muscle without cooking it enough to kill the larval tapeworm, the tapeworm sets up shop in the human's intestine, grows to full size, and releases eggs in the feces. Larval tapeworms can also go on vacation in humans and end up forming cysts in muscles or even the brain or other organs.

- *Diphyllobothrium* eggs hatch in water and are eaten by small crustaceans, which then are eaten by small fish, which in turn are eaten by large fish, where the immature tapeworm larvae end up as cysts in the muscles. If a human eats undercooked fish muscle, the tapeworm once again goes to town in the human's intestine.

- *Hymenolepis* eggs hatch in insects or rodents, and the larvae may be ingested by humans eating food that has been contaminated by those insects or rodents. Once in humans, they set up shop in the intestine as usual.

- *Echinococcus* eggs are ingested by and hatch in sheep or other herbivorous mammals, form larval cysts in muscle and other tissues, get eaten by dogs or other carnivorous mammals, mature in the intestine, and produce fertilized eggs that are excreted in feces to repeat the process. Humans who accidentally ingest eggs from carnivore feces may have tapeworm larvae migrating through their bloodstream and forming cysts in almost any of their organs, but mature tapeworms generally do not reach the human intestine and so cannot reproduce in humans.

7.2 Anti-Helminth Therapeutics

Helminths are multicellular animals and we are multicellular animals, which really limits the options for treatment. The trick is to find therapeutics that are absorbed more by helminths than by their human host. Existing therapeutics tend to fall into four major categories:

(a) Microtubule inhibitors bind to beta-tubulin to block cytoskeleton growth and movement. They are especially useful against most nematodes, some trematodes, and some cestodes. Some major examples include:

- Albendazole
- Mebendazole
- Thiabendazole
- Triclabendazole

(b) Calcium drugs alter membrane permeability to calcium ions and thereby paralyze helminths. They are especially useful against many trematodes and cestodes. The main flavors are:

- Praziquantel
- Pyrantel pamoate
- Oxamniquine (mainly useful against *Schistosoma* blood flukes)

(c) GABA agonists activate gamma-amino-butyric-acid-gated or glutamate-gated chloride ion channels in muscles to paralyze helminths. They are effective against some nematodes and trematodes. In some cases the patient's stool contains partially paralyzed but still alive worms, which is always lovely. Examples include:

- Ivermectin
- Piperazine
- Diethylcarbamazine

(d) Metabolic drugs impair nutrient metabolism and energy conversion in helminths. Currently the major example is:

- Niclosamide is effective against many cestodes, acting to inhibit their glucose metabolism.

7.3 Useful Applications of Helminths

Not all helminths are slimy parasites that want to eat your body from the inside out. Some of them are really very nice and polite. Some useful application of helminths include:

- Most parasitic helminths have mechanisms to evade and/or suppress the host's immune system, in order to prevent the immune system from destroying the worms, their eggs, and their larvae. For that reason, in some cases helminth infection can suppress excessive immune responses that are associated with autoimmune diseases, asthma or allergies, or infertility/loss of pregnancies due to immune attacks on the human fetus. Of course, most people aren't eager to volunteer to be infected with helminths, but scientific study of molecules coating or secreted by helminths may lead to useful new drugs to suppress excessive immune responses.

- Likewise, some people have advocated helminths for weight loss, but most folks aren't eager to sign up and even jogging looks more fun.

- Many types of worms are important for agriculture, from freeliving (nonparasitic) earthworms that improve the soil to species of nematodes that only infect insects and can be used as natural insecticides.

- *Caenorhabditis elegans* is a nonpathogenic nematode that lives free in water, grows to be approximately 1 mm long, and is widely used as the simplest model for multicellular animal development and genetics. Many genes and functions that were first studied in *C. elegans* were ultimately found to have closely related cousins in other species, even humans. For example, discovery of the simple version of the apoptosis pathway in *C. elegans* led to the discovery of the homologous but more complex apoptosis pathway (Fig. 6) in humans and other animals.

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