MICROBIAL GENETICS

CHAPTER 7

Courtesy Karen Nelson/Tigr

A fair amount of the material in this chapter is repeat stuff you have had. Because of the importance of the material in chapters 7 and 8 I will cover it. Please bear with me.

Microbial Genetics

- Heredity
- Chromosomes
- Genes
- Alleles

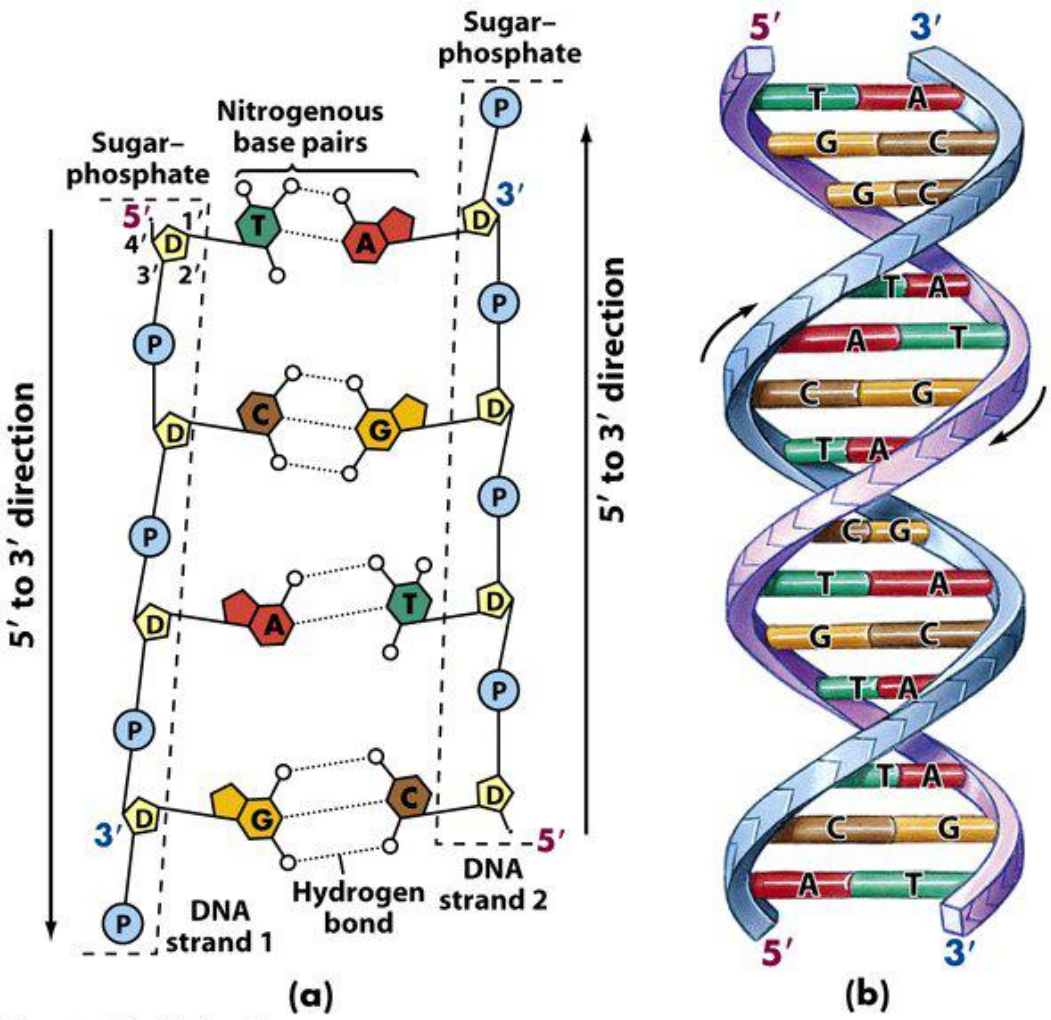

Figure 7-1 Microbiology, 6/e
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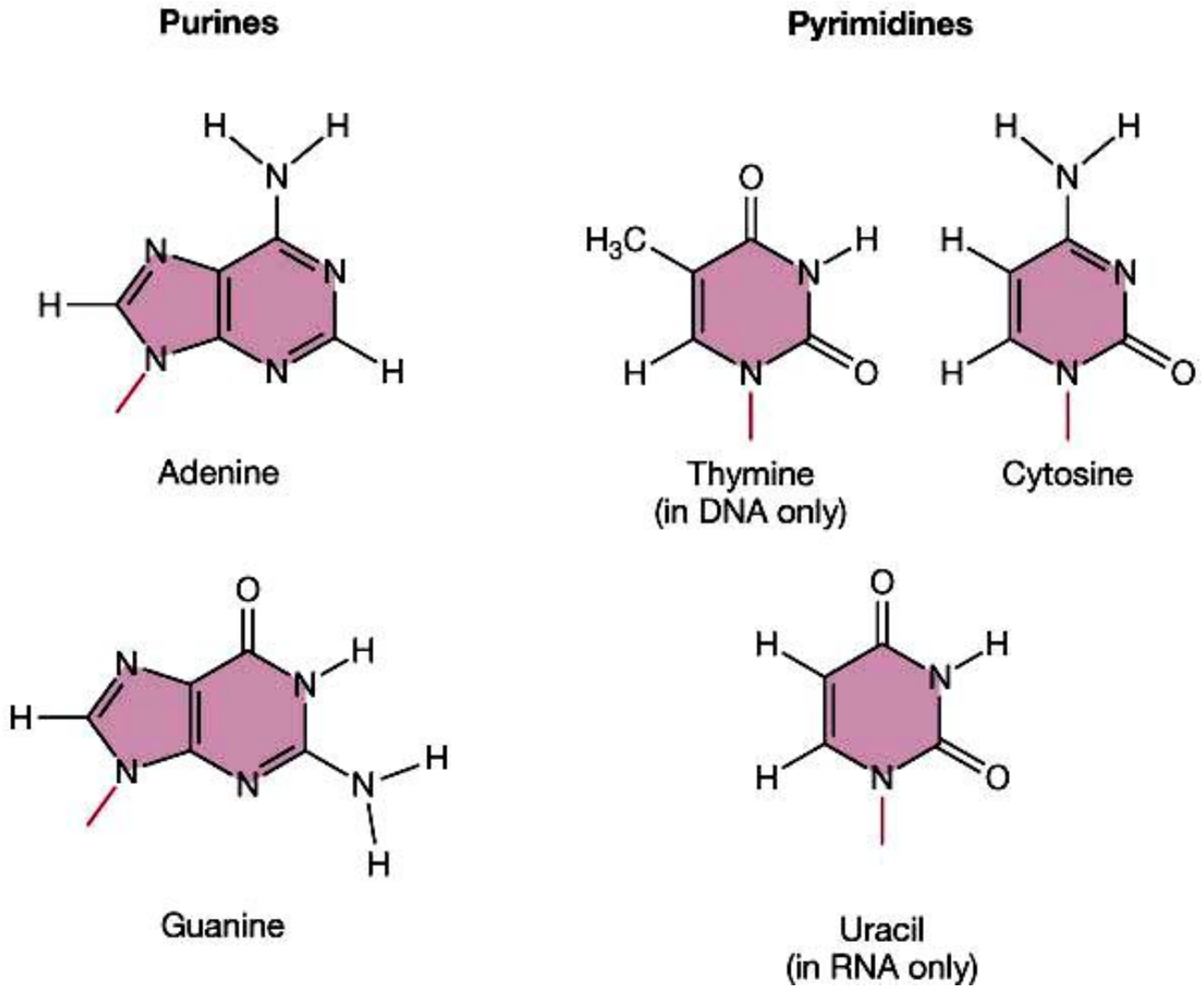
Two strands are held together by hydrogen bonding. A-T; G-C pair. If G were opposite T they would not hydrogen bond and you would get a bulge or bubble in the DNA (important in both repair and in DNA synthesis when the wrong nucleotide is accidentally added). The strands are **antiparallel and the DNA molecule is twisted into a double helix.** The two sugar-phosphate strands run in opposite **(antiparallel)** directions. Each new strand grows from the 5' end toward the 3' end **(that is, nucleotides are added to the 3' end).**

http://www.youtube.com/watch?v=qy8dk5iS1f0&feature=related

The web site above gives a basic animation of the structure of DNA- probable redundant for most of you

Fig. 7.1 The structure of DNA



You will be responsible for recognizing the different bases found in nucleic acids

Figure 2.22 The five bases found in nucleic acids

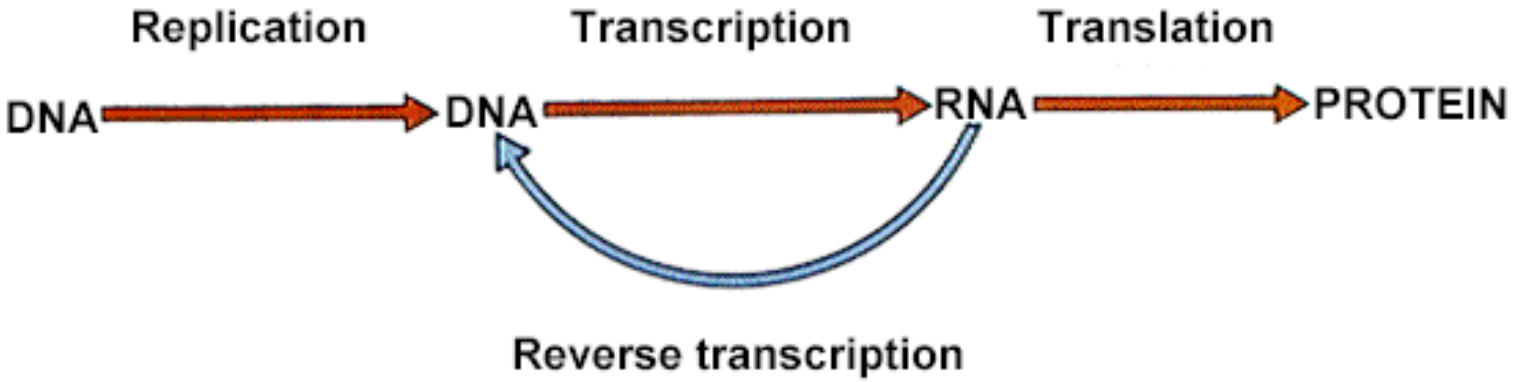
Brain Implant Improves Thinking in Monkeys, First Such Demonstration in Primates Scientists have designed a brain implant that sharpened decision making and restored lost mental capacity in monkeys, providing the first demonstration in primates of the sort of brain prosthesis that could eventually help people with damage from dementia, strokes or other brain injuries. Previous studies have shown that a neural implant can do this for memory in rodents, but the new report extends that work significantly, experts said — into brains that are much closer to those of humans. In the study, researchers at Wake Forest trained five rhesus monkeys to play a picture-matching game. The monkeys saw an image on a large screen — of a toy, a person, a mountain range — and tried to select the same image from a larger group of images that appeared on the same screen a little while later. The monkeys got a treat for every correct answer. After two years of practice, the animals developed some mastery, getting about 75 percent of the easier matches correct and 40 percent of the harder ones, markedly better than chance guessing. The monkeys were implanted with a tiny probe with two sensors; it was threaded through the forehead and into two neighboring layers of the cerebral cortex, the thin outer covering of the brain. To test the device, the team relayed this "correct" signal into the monkeys' brains when they were in the middle of choosing a possible picture match, and it improved their performance by about 10 percent.

The researchers then impaired the monkeys' performance deliberately, by dosing them with cocaine. Their scores promptly fell by 20 percent. But when you turn on the stimulator, they don't make those errors; in fact, they do a little better than normal," said Robert E. Hampson of Wake Forest, a study author. His co-authors were Sam A. Deadwyler, Ioan Opris and Lucas Santos, all of Wake Forest; Dr. Berger, Vasilis Marmarelis and Dong Song of U.S.C.; and Greg A. Gerhardt of the University of Kentucky. The technology used in the study could easily be contained on an implantable chip, Dr. Deadwyler said, and it is possible to envision a system that could help people with brain damage.

Prolonged CPR Holds Benefits, a Study Shows When a hospital patient goes into cardiac arrest, one of the most difficult questions facing the medical team is how long to continue cardiopulmonary resuscitation. Now a new study involving hundreds of hospitals suggests that many doctors may be giving up too soon. The study found that patients have a better chance of surviving in hospitals that persist with CPR for just nine minutes longer, on average, than hospitals where efforts are halted earlier. There are no clear, evidence-based guidelines for how long to continue CPR efforts. The findings challenge conventional medical thinking, which holds that prolonged resuscitation for hospitalized patients is usually futile because when patients do survive, they often suffer permanent neurological damage. To the contrary, the researchers found that patients who survived prolonged CPR and left the hospital fared as well as those who were quickly resuscitated. The study, published online Tuesday in The Lancet, is one of the largest of its kind and one of the first to link the duration of CPR efforts with survival rates. It should prompt hospitals to review their practices and consider changes if their resuscitation efforts fall short, several experts said. One of the first surprises was the significant variation in duration of CPR among the hospitals, ranging from a median of 16 minutes in hospitals spending the least amount of time trying to revive patients to a median of 25 minutes among those spending the most — a difference of more than 50 percent.

The researchers initially thought they would find that some patients were being subjected to protracted resuscitation efforts in vain, said the senior author, Dr. Brahmajee Nallamothu, an associate professor at the University of Michigan and a cardiologist at the Ann Arbor VA Medical Center.

But as it turned out, those extra minutes made a positive difference. Patients in hospitals with the longest CPR efforts were 12 percent more likely to survive and go home from the hospital than those with the shortest times.



Three methods of information transfer:

1. **Replication**- DNA makes new DNA
2. **Transcription**- DNA makes RNA-initial step in protein synthesis and synthesis of ribonucleoproteins (such as ribosomes) and transfer RNA.
3. **Translation**- RNA links amino acids together to form proteins

In both DNA replication and transcription , DNA serves as a template for synthesis of a new nucleotide polymer. The sequence of bases in each polymer is complementary to that in the original DNA. In RNA, thymine is replaced by uracil, which pairs with adenine.

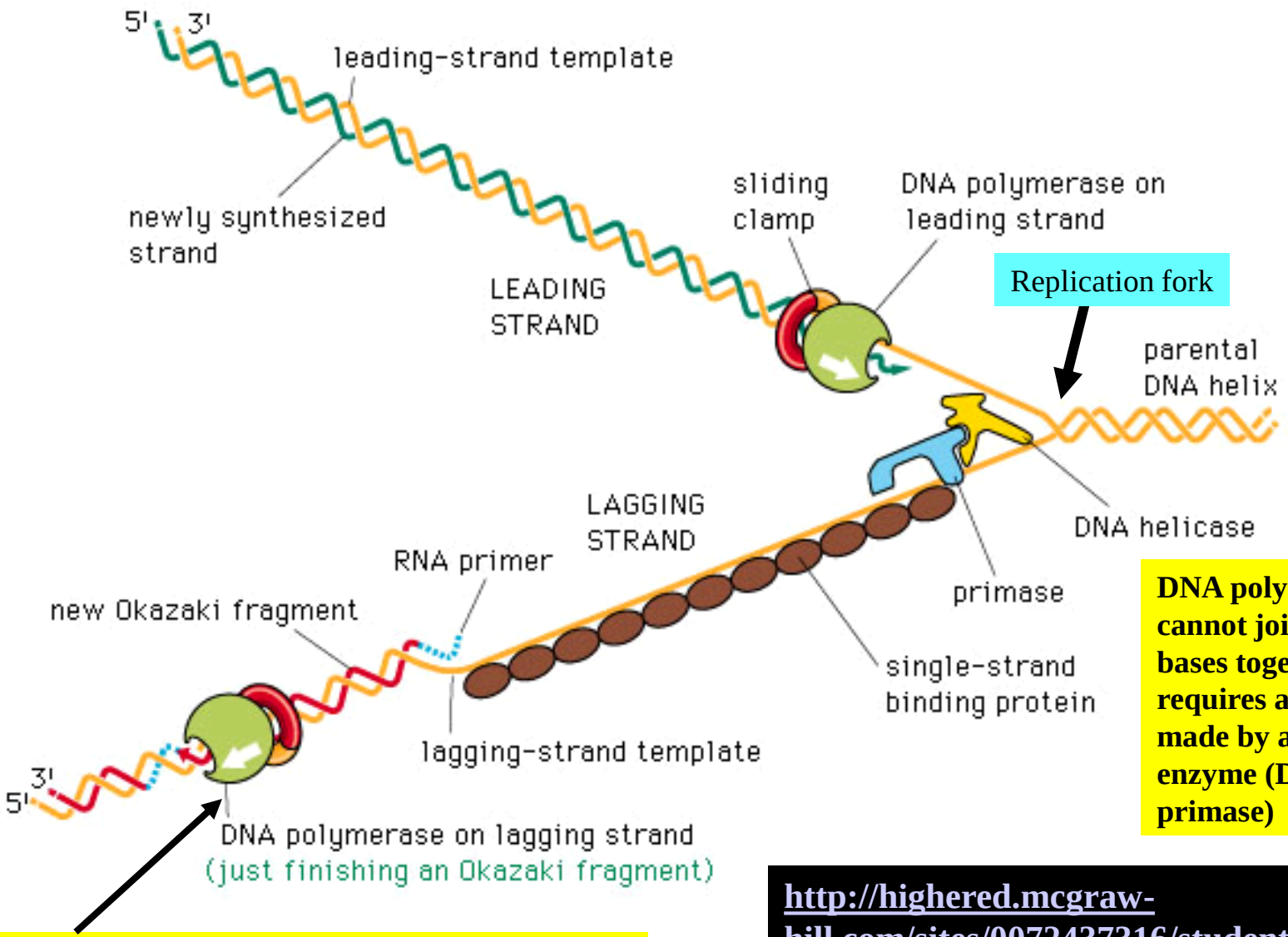
DNA replication, transcription, and translation all transfer information from one molecule to another. These processes allow information in DNA to be transferred to each new generation of cells and to be used to control the functioning of cells.

Fig. 7.3 **The transfer of information from DNA to protein**

http://highered.mcgraw-hill.com/sites/0072437316/student_view0/chapter14/animations.html

How DNA nucleotides are added during DNA replication

Above website gives an animated overview of DNA replication



DNA polymerase cannot joint 2 DNA bases together it requires a primer made by another enzyme (DNA primase)

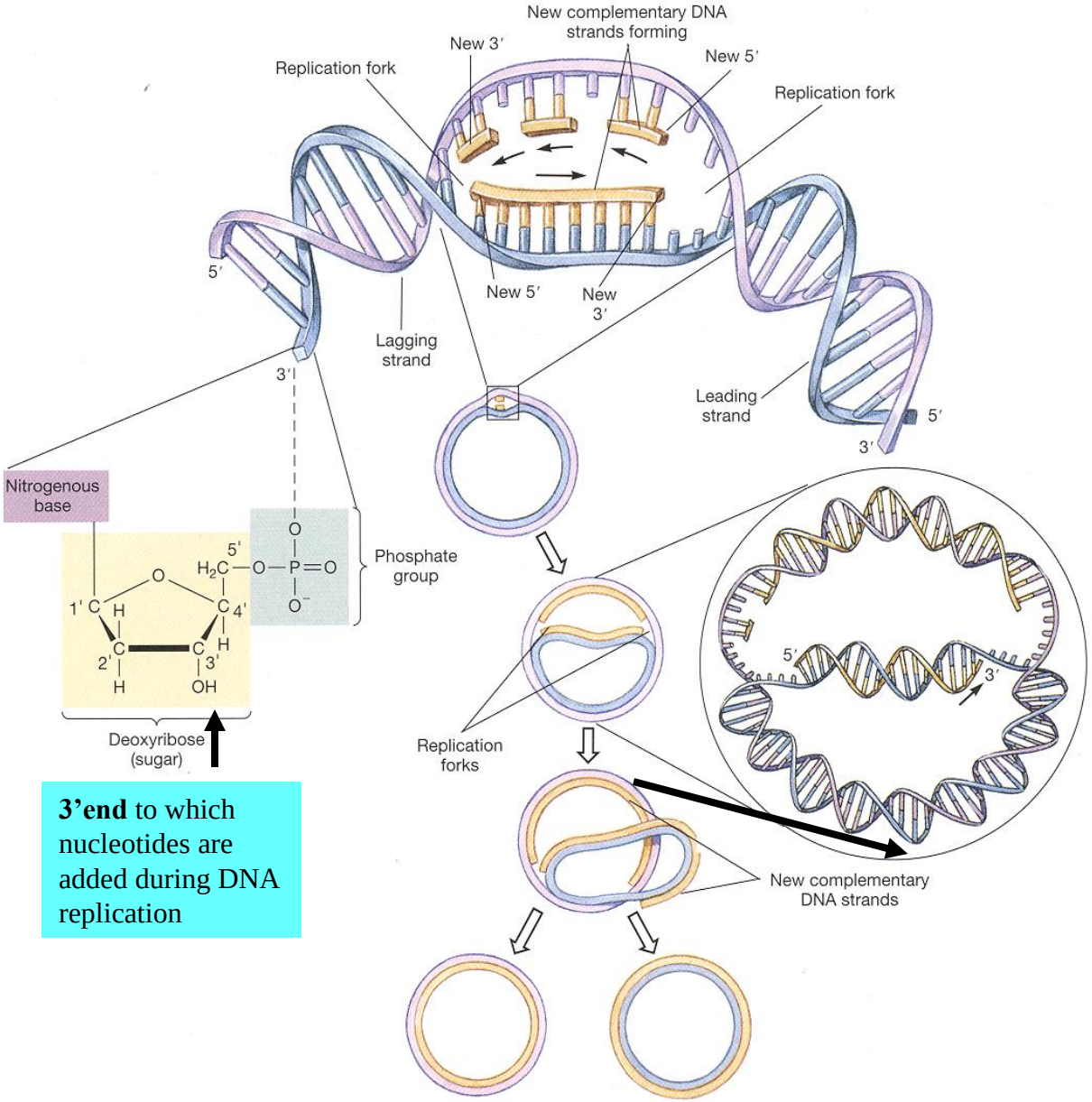
sliding clamp and DNA polymerase fall off the DNA and reattach at the point of synthesis of the newly replicated Okazaki fragment.

http://highered.mcgraw-hill.com/sites/0072437316/student_view0/chapter14/animations.html

DNA replication fork

Fig. 7.4b (this figure is not in your text but the process is described in your text). It is effectively a close-up look at Fig. 7.4.

- DNA replication in procaryote- DNA strands separate, and replication begins at a replication fork on each strand. As synthesis proceeds, each strand of DNA serves as template for the replication of its partner. The strands are antiparallel.



3'end to which nucleotides are added during DNA replication

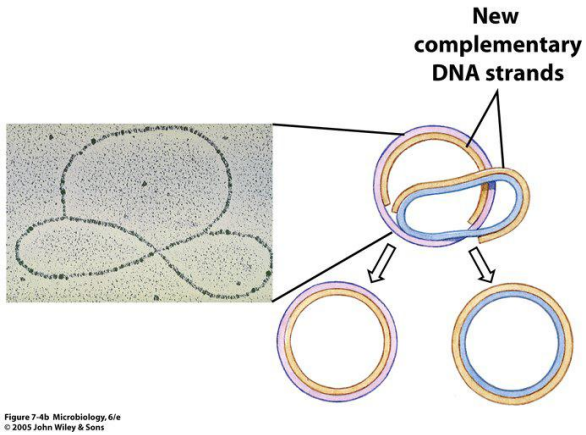
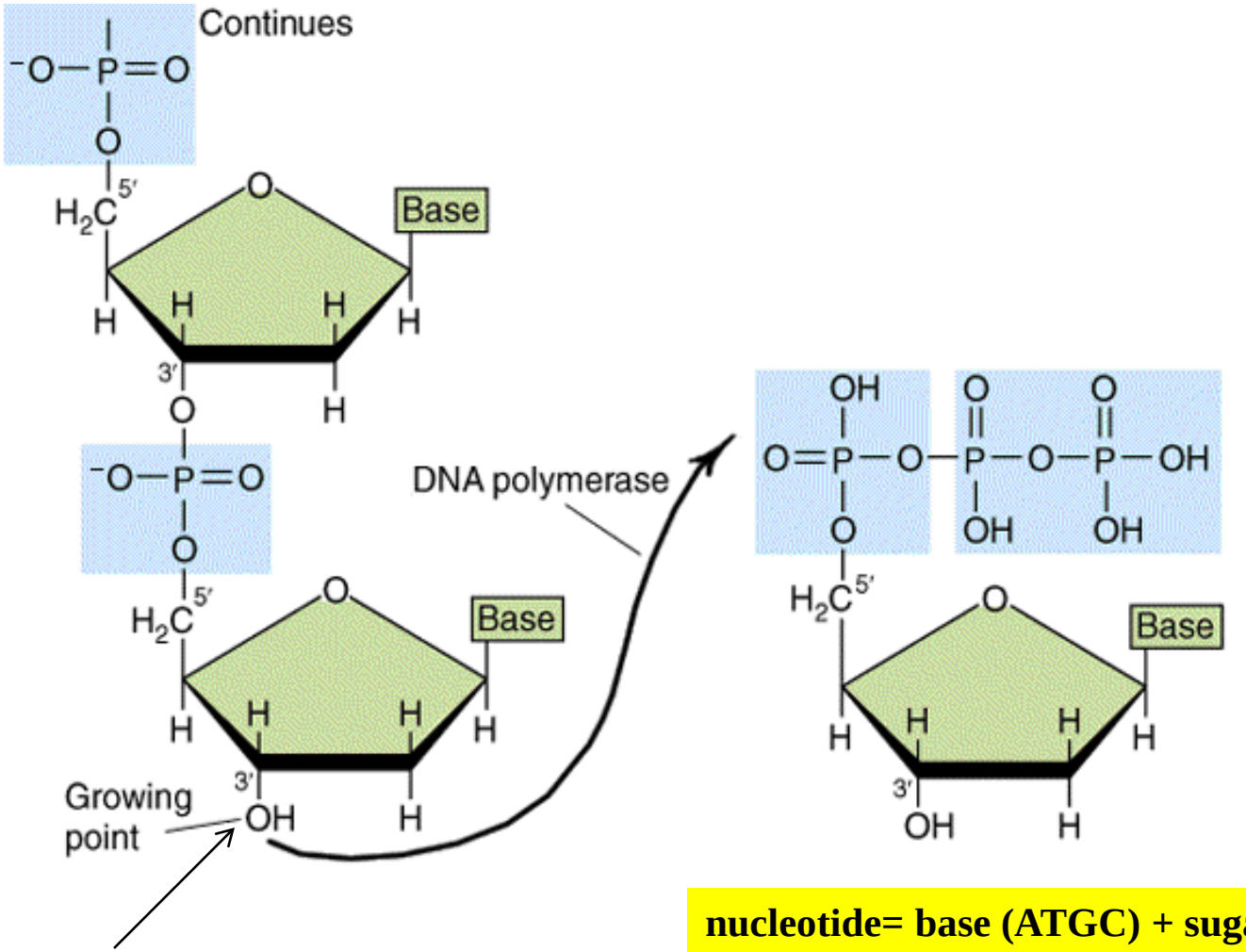


Figure 7-4b Microbiology, 6/e © 2005 John Wiley & Sons

Fig. 7.4 DNA replication in a prokaryote



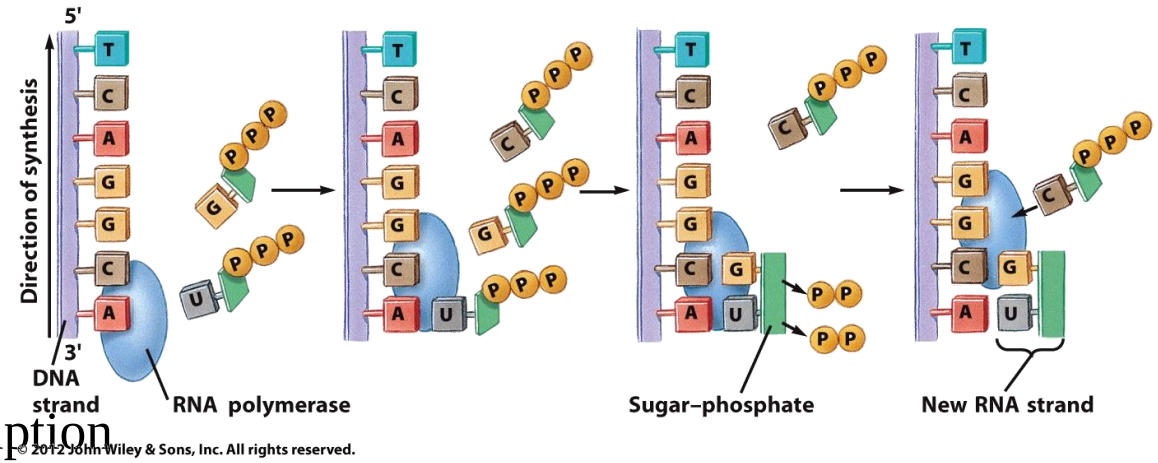
3' carbon of deoxyribose

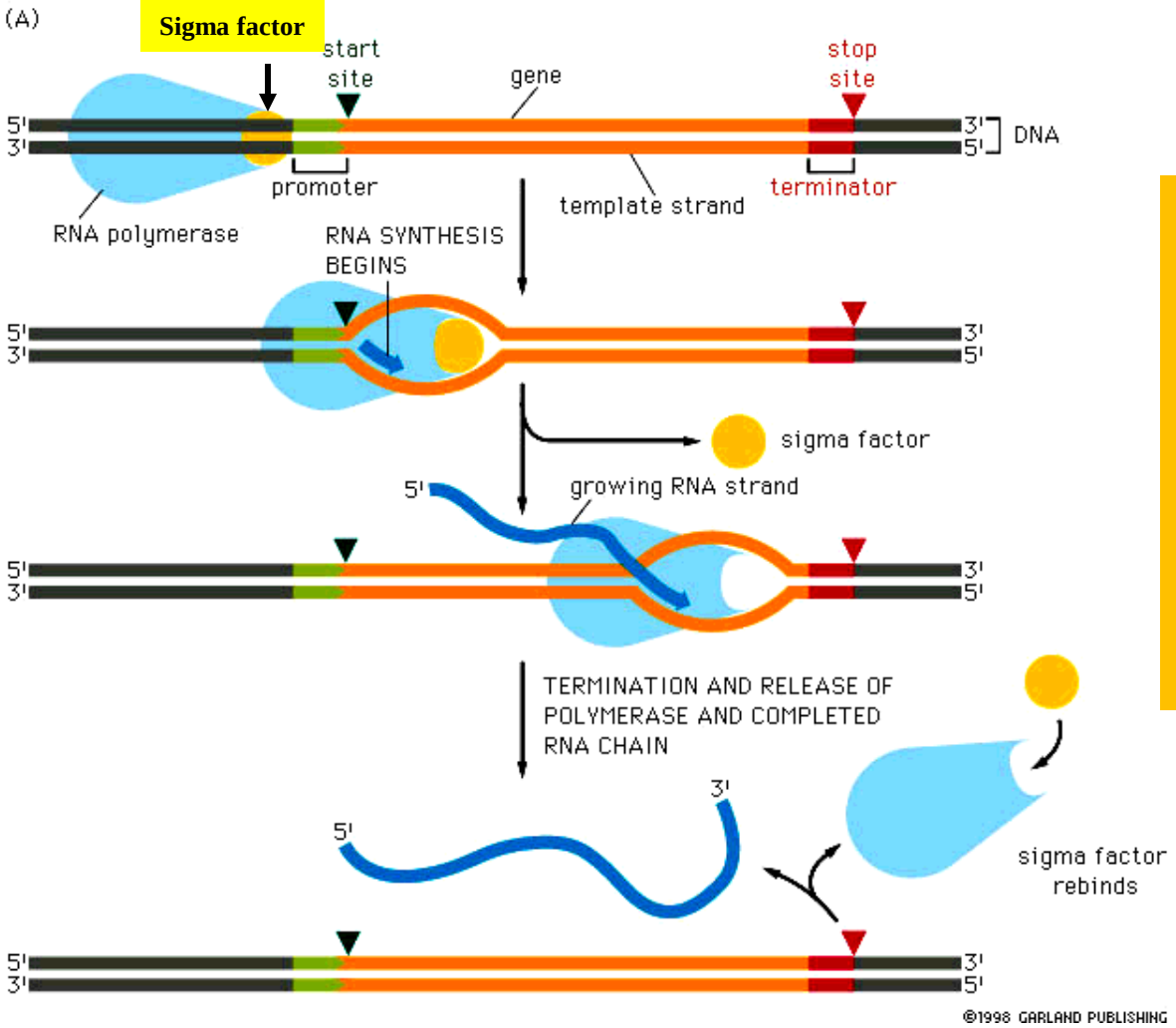
nucleotide= base (ATGC) + sugar (deoxyribose + one or more phosphates)

Nucleotides are added to the 3' end of the DNA-hence synthesis is said to be in a 5' to 3' direction.

Transcription

- DNA to RNA
- **One strand only serves as RNA template**
- RNA polymerase
- Promoter
- Simultaneous transcription and translation





This figure is of prokaryotic transcription

In eukaryotic cells there are a number general transcription factors instead of the sigma factor

RNA polymerase binds to one and only one strand of exposed DNA-termed the "sense" strand. More specifically the RNA polymerase binds to a region of the sense strand called the **promoter region. In prokaryotes, transcription and translation both take place in the cytoplasm whereas in eukaryotes, transcription takes place in the nucleus.**

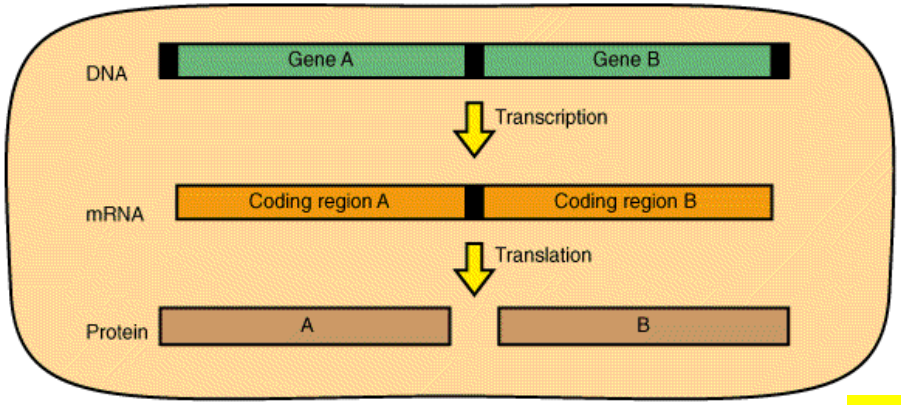
Fig. 7.5 The transcription of RNA from template DNA. Sigma factor must be attached to the RNA polymerase to begin transcription. The RNA polymerase binds to a region "upstream of the start site of transcription

Transcription- All cells must constantly synthesize proteins to carry out their life processes: reproduction, growth repair and regulation of metabolism

This animation gives an overview of prokaryotic versus eukaryotic gene expression, i.e. transcription

http://highered.mcgraw-hill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120077/bio25.swf::Processing of Gene Information - Prokaryotes versus Eukaryotes

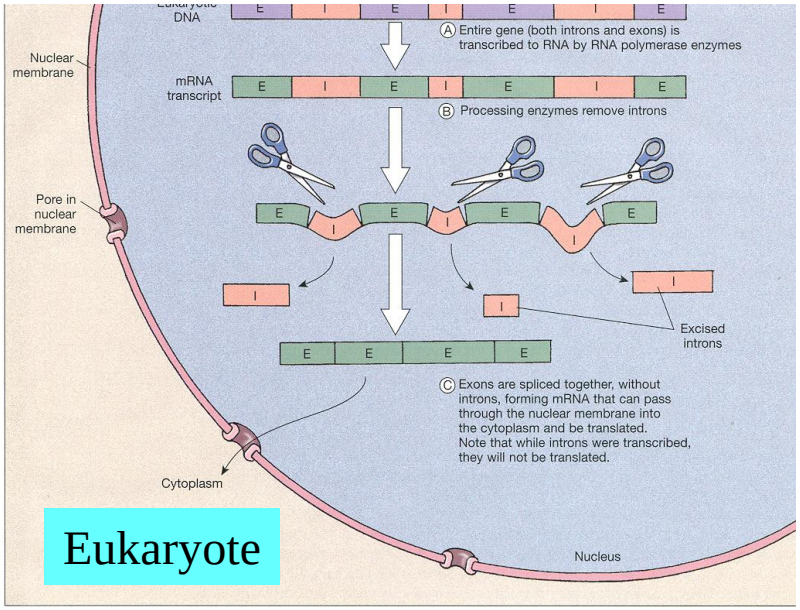
View the above on your own the figures below basically cover the same material



PROKARYOTE

In prokaryotic cells one mRNA molecule corresponds to one or <u>more</u> genes and there are no introns

In both the pro- and eukaryotic genes RNA polymerase binds, at the promoter region (which involves TATA binding region), to only one strand of exposed DNA-termed the "sense" strand.



Eukaryote

In eukaryotic genes one mRNA corresponds to only one gene and there are introns. However alternative splicing can result in more than 1 protein/gene.

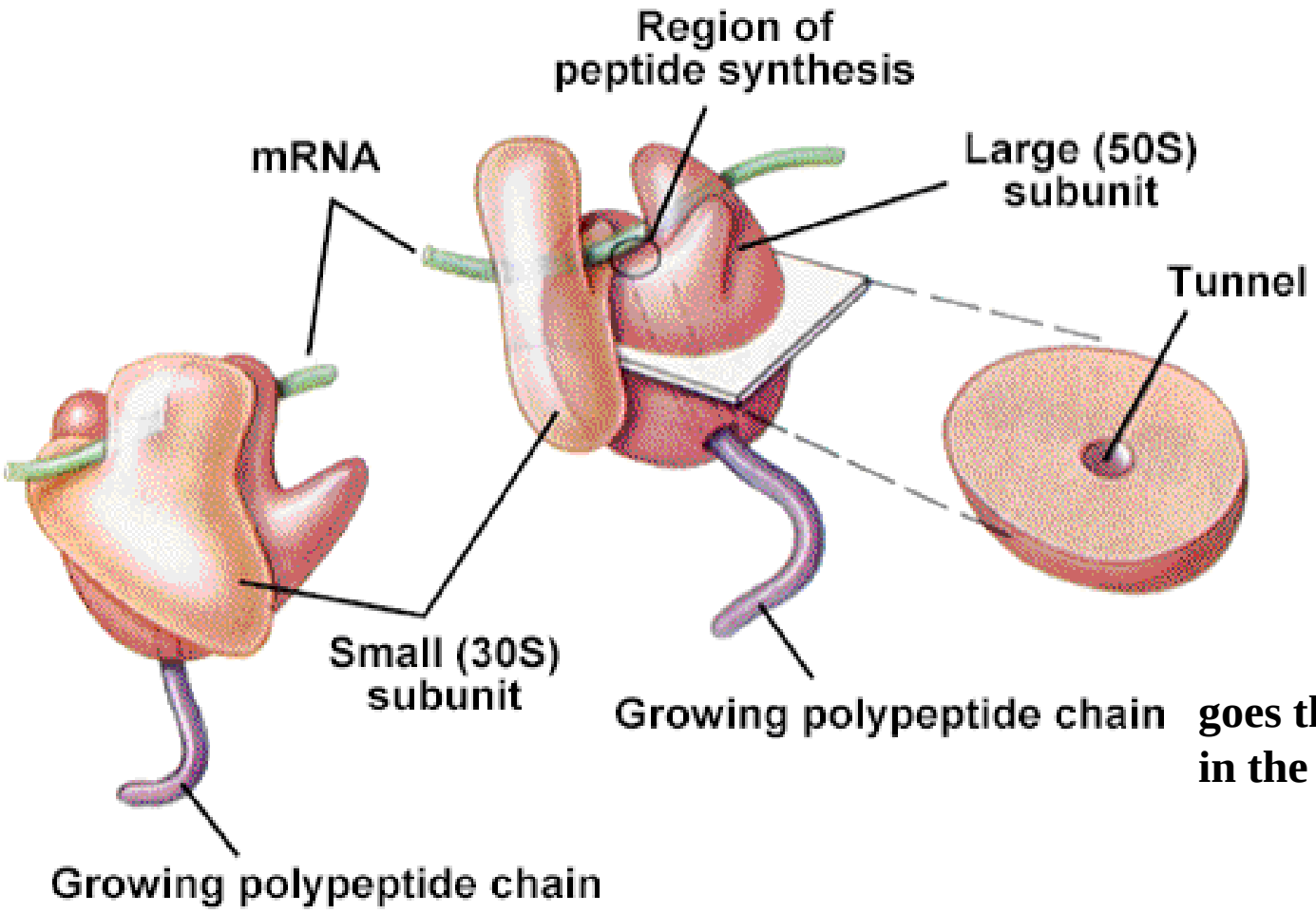
Fig. 7.6 **Eukaryotic genes differ in complexity from prokaryotic genes**

Pneumonia bug evolves to evade vaccine: study Bugs that cause childhood pneumonia and meningitis have evolved to evade vaccines by swapping bits of their genome with other bacteria, according to a study published Sunday. The findings, published in Nature Genetics, show how quickly these life-threatening pathogens can disguise themselves with borrowed genetic decoys, and how hard it is for medicine to keep up. Diseases caused by Streptococcus pneumoniae are thought to kill over a million young children around the world each year. Vaccines that protect against these so-called pneumoccoccal infections are designed to recognise a material on the outer surface of a bacterium's cell called polysaccharide. Each of over 90 kinds, or "serotypes", of these bacteria have a different polysaccharide coating. 2000, a vaccine that targeted seven serotypes proved highly effective when introduced in the United States. The same formula -- which also prevented transmission from children to adults -- was adopted in Britain. Over time, however, the vaccine worked less well, so researchers led by Rory Bowden at the University of Oxford set out to discover why. Combining cutting-edge genetic analysis with epidemiology, which examines how disease spreads, they found that the deadly pathogens escaped detection by swapping genes with other, slightly different, bacteria. The current vaccine strategy ... is extremely effective," co-author Bernard Beall, a scientist at the Centers for Disease Control and Prevention in Atlanta, said in a statement.

"However, our observations indicate that the organism will continue to adapt to this strategy with some measurable success."

Fatal listeria outbreak linked to imported cheese **A national outbreak of listeria linked to imported cheese has killed at least one person and sent 14 to the hospital, the Centers for Disease Control and Prevention said. Three deaths have been reported among those who were sickened but so far listeria has been confirmed as a contributing factor in only one of those. CDC and state officials are investigating the other deaths. Forever Cheese of Astoria, N.Y., has voluntarily recalled one lot of its Frescolina-brand ricotta salata cheese because of *Listeria monocytogenes* contamination. The first person fell ill on March 28, the most recent on Aug.30, according to CDC. All 14 ill individuals were hospitalized. Four of those sickened were pregnant women, two were newborns. The other 10 people were between 56 and 87 years old. Listeriosis, the illness caused by infection with listeria monocytogenes, is serious and can be deadly. Symptoms include fever and muscle aches, sometimes preceded by diarrhea or other gastrointestinal symptoms. Victims can also have headache, stiff neck, confusion, loss of balanceand convulsions. In pregnant women, listeriosis is especially dangerous because, although it may cause only a mild, flu-like illness in the mother, it can lead to miscarriage, stillbirth, premature delivery or life-threatening infection of the newborn. It is also very dangerous to the elderly and adults with weakened immune systems.**

Bits of Mystery DNA, Far From 'Junk,' Play Crucial Role Among the many mysteries of human biology is why complex diseases like diabetes, high blood pressure and psychiatric disorders are so difficult to predict and, often, to treat. An equally perplexing puzzle is why one individual gets a disease like cancer or depression, while an identical twin remains perfectly healthy. Now scientists have discovered a vital clue to unraveling these riddles. The human genome is packed with at least four million gene switches that reside in bits of DNA that once were dismissed as "junk" but that turn out to play critical roles in controlling how cells, organs and other tissues behave. The discovery, considered a major medical and scientific breakthrough, has enormous implications for human health because many complex diseases appear to be caused by tiny changes in hundreds of gene switches. As scientists delved into the "junk" — parts of the DNA that are not actual genes containing instructions for proteins — they discovered a complex system that controls genes. At least 80 percent of this DNA is active and needed. The result of the work is an annotated road map of much of this DNA, noting what it is doing and how. It includes the system of switches that, acting like dimmer switches for lights, control which genes are used in a cell and when they are used, and determine, for instance, whether a cell becomes a liver cell or a neuron. The discoveries were published on Wednesday in six papers in the journal Nature and in 24 papers in Genome Research and Genome Biology. In addition, The Journal of Biological Chemistry is publishing six review articles, and Science is publishing yet another article. In one of the Nature papers, researchers link the gene switches to a range of human diseases — multiple sclerosis, lupus, rheumatoid arthritis, Crohn's disease, celiac disease — and even to traits like height. In large studies over the past decade, scientists found that minor changes in human DNA sequences increase the risk that a person will get those diseases. But those changes were in the junk, now often referred to as the dark matter — they were not changes in genes — and their significance was not clear. The new analysis reveals that a great many of those changes alter gene switches and are highly significant. "Most of the changes that affect disease don't lie in the genes themselves; they lie in the switches," said Michael Snyder, a Stanford University researcher for the project, called Encode, for Encyclopedia of DNA Elements.



goes through a tunnel in the 50S subunit

The **small (30S) and large (50S) subunits** are shown from two different angles. The subunits enfold the mRNA strand. The region of peptide synthesis is the junction of these three compoenents. The growing polypeptide chain passes through a tunnel in the 50S subunit, which can be seen in cross-section. Eukaryotic ribosomes are comprised of a **60S and a 40S subunit,**

Fig. 7.7 **Prokaryotic ribosomal structure**

RNA Types

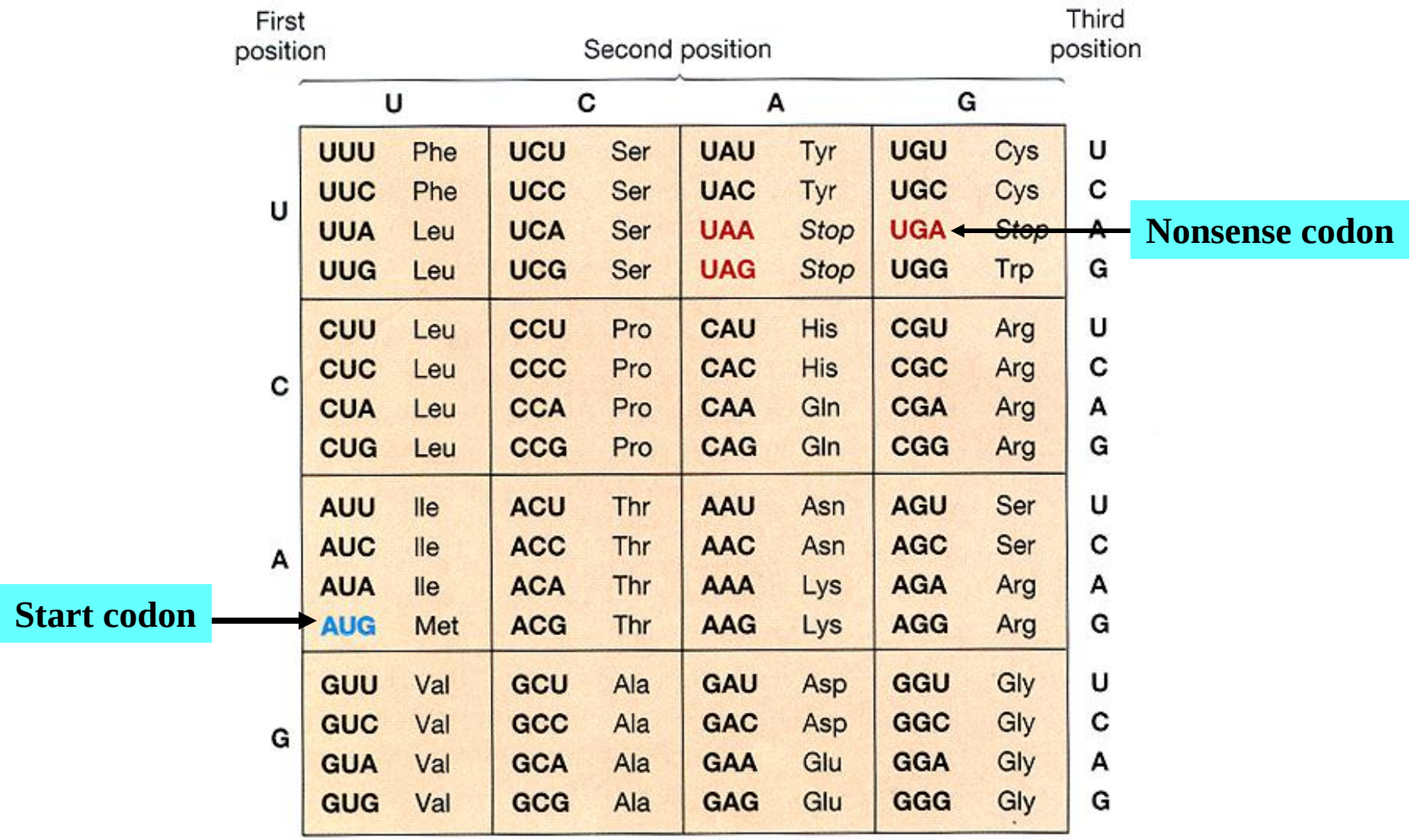
- Ribosomal RNA (rRNA)
- Messenger RNA (mRNA)
- Transfer RNA (tRNA)

Messenger RNA (mRNA). In prokaryotic cells one mRNA molecule corresponds to one or more genes (in eukaryotes one mRNA corresponds to one gene). Each mRNA molecule becomes associated with one or more ribosomes. At the ribosome, the information coded in mRNA acts during translation to dictate the sequence of amino acids in the protein.

In translation each triplet (sequence of three bases) in mRNA constitutes a codon. Codons are the "words" in the language of nucleic acids. Each codon specifies a particular amino acid or acts as a terminator codon.

Start codon is the first codon in the mRNA and is the codon AUG for methionine in eukaryoties and formylmethionine in prokaryotes.

The last codon to be translated in a molecule of mRNA is a terminator, or stop codon. It causes the RNA polymerase to fall off the mRNA.

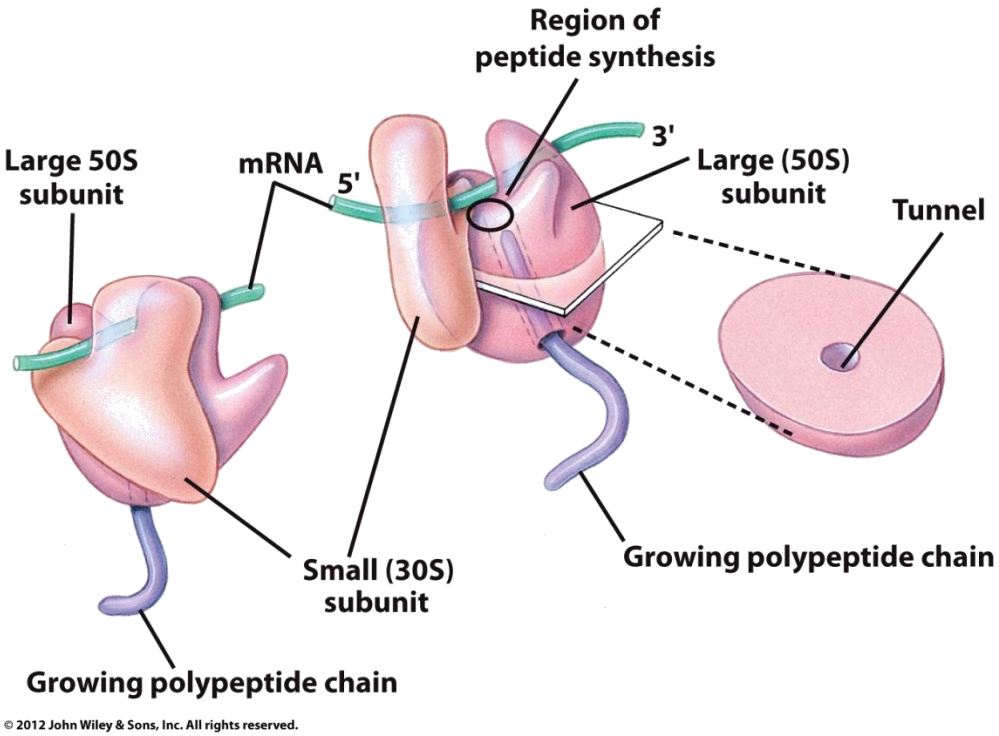


In *Mycoplasma sp.* *UGA codes for tryptophan-* we (in my lab.) are trying to express a mycoplasmal gene (arginine deiminase) that has seven UGA's in *Escherichia coli-(the above is the genetic code that E.coli uses)* what do we need to do???

Fig. 7.8 The genetic code, with standard three-letter abbreviations for amino acids

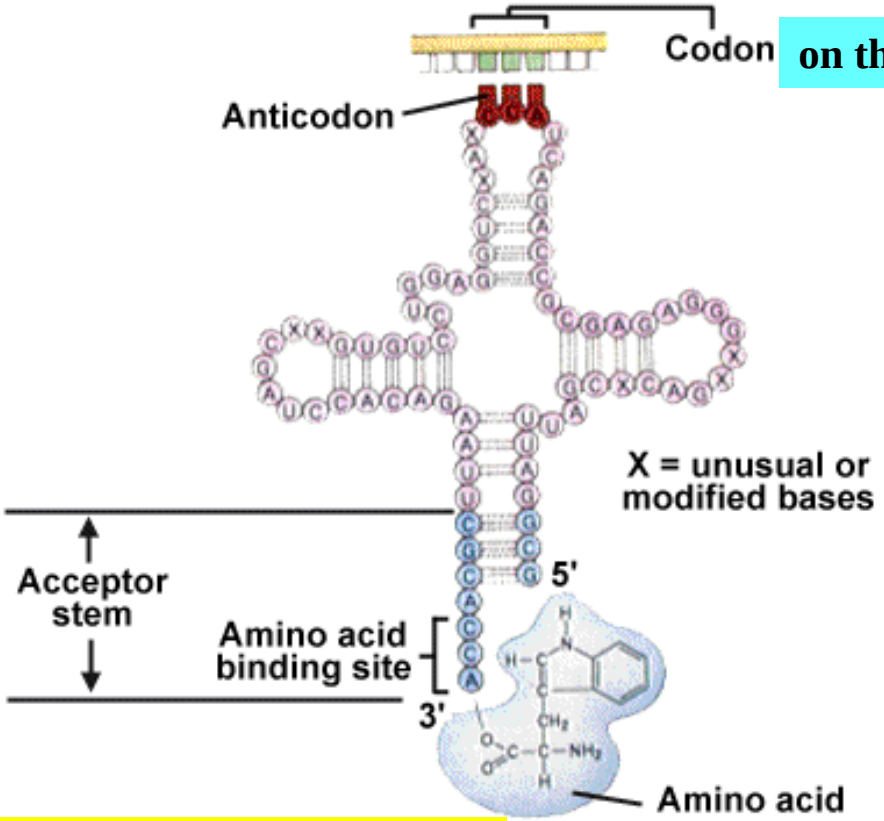
Translation

- mRNA codons
- tRNA anticodons
- Amino acid links
- Role of ribosomes



Transfer RNA (tRNA)

The function of transfer RNA (tRNA) is to transfer amino acids from the cytoplasm to the ribosomes for placement in a protein molecule. Many different kinds of tRNA's have been isolated from the cytoplasm of cells. Each tRNA has a three-base anticodon that is complementary to a particular mRNA codon



The two-dimensional structure of the tryptophan transfer RNA**.** The amino acid attaches to CCA (all tRNA's have the same amino acid attachment site)

http://www.youtube.com/watch?v=T54Akbu0ONU

Amino acids are attached to the tRNA by aminoacyl-tRNA synthetase. Only the anticodon/codon is involved in the recognition of the tRNA and not the amino acid.

Fig. 7.9 **Transfer RNA**

Another class of RNA's have been discovered and are termed RNAi

The Mechanism of RNA Interference (RNAi)

Long double-stranded RNAs (dsRNAs; typically >200 nt) can be used to silence the expression of target genes in a variety of organisms and cell types (e.g., worms, fruit flies, and plants). In mammalian cells, introduction of long dsRNA (>30 nt) initiates a potent antiviral response, exemplified by nonspecific inhibition of protein synthesis and RNA degradation. The implication for use of RNAi in disease therapy are amazing and several biotech companies have been established just to exploit these possibilities.

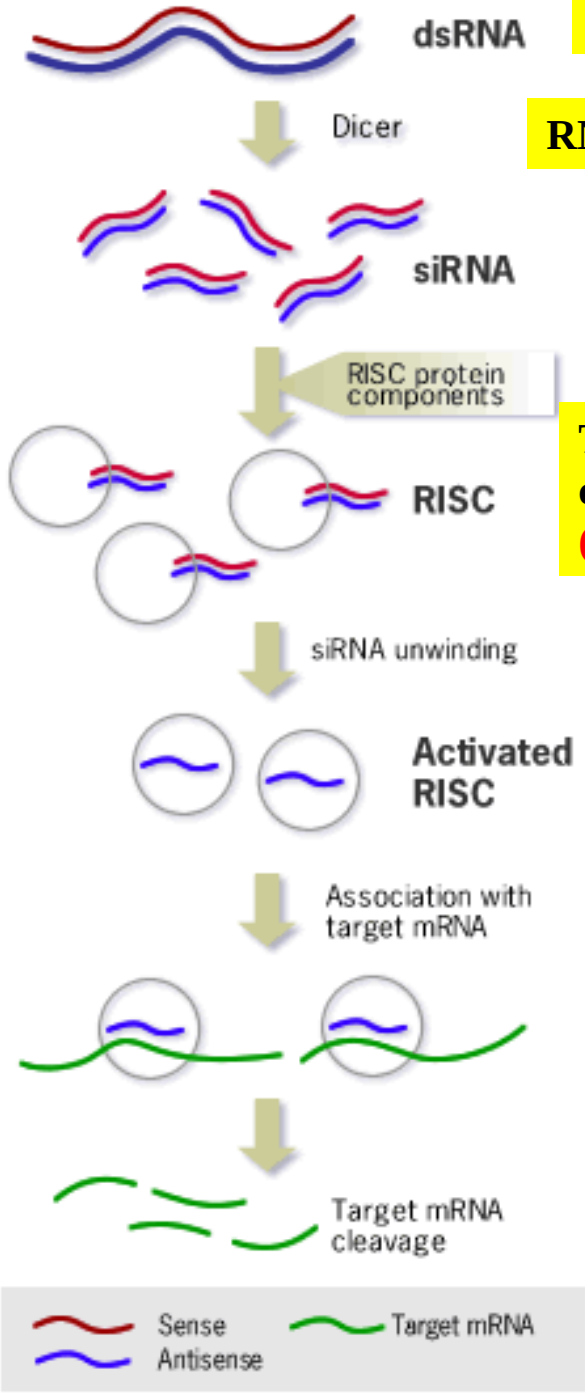
http://www.youtube.com/watch?v=e2dnFBnFWT8&feature=related

Shorter version

RNAi

http://www.youtube.com/watch?v=UdwygnzIdVE&feature=related

Longer version



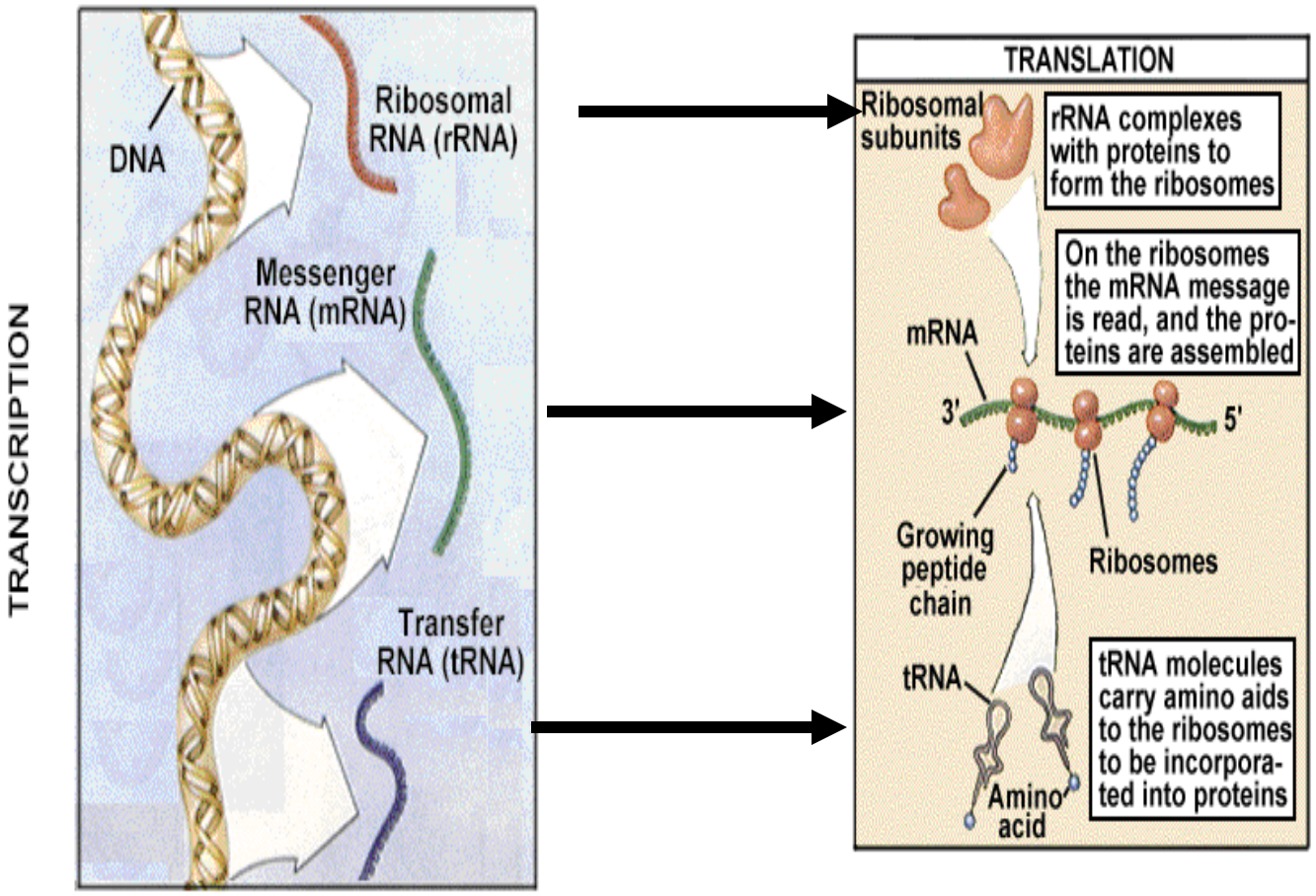
Introduction of a long dsRNA

RNase III-like enzyme called Dicer (initiation step).

Then, the siRNAs assemble into endoribonuclease-containing complexes known as RNA-induced silencing complexes (RISCs), unwinding in the process.

The siRNA strands subsequently guide the RISCs to complementary RNA molecules, where they cleave and destroy the cognate RNA (effecter step). Cleavage of cognate RNA takes place near the middle of the region bound by the siRNA strand.

The Mechanism of RNA Interference (RNAi)



The three types of RNA: rRNA, mRNA, and tRNA

RNAi is another type of functional RNA

Fig. 7.10 **Transcription and translation**

TABLE 7.1

Properties of the Different Kinds of RNA

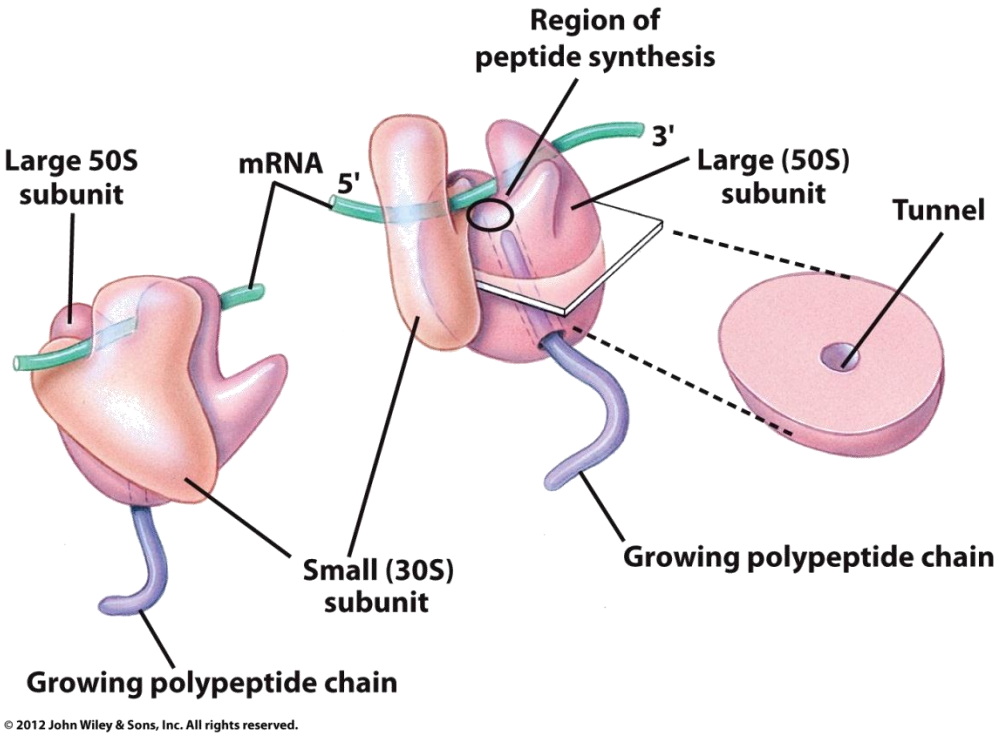
Kind of RNA	Properties
Ribosomal	Combines with specific proteins to form ribosomes.
	Serves as a site for protein synthesis.
	Associated enzymes function in controlling protein synthesis.
Messenger	Carries information from DNA for synthesis of a protein.
	Molecules correspond in length to one or more genes in DNA.
	Has base triplets called codons that constitute the genetic code.
	Attaches to one or more ribosomes.
Transfer	Found in the cytoplasm, where they pick up amino acids and transfer them to mRNA.
	Molecules have a cloverleaf shape with an attachment site for a specific amino acid.
	Each has a single triplet of bases called an anticodon, which pairs complementarily the corresponding codon in mRNA.

Table 7-1 Microbiology, 6/e

RNAi is another type of functional RNA

Translation

- mRNA codons
- tRNA anticodons
- Amino acid links
- Role of ribosomes

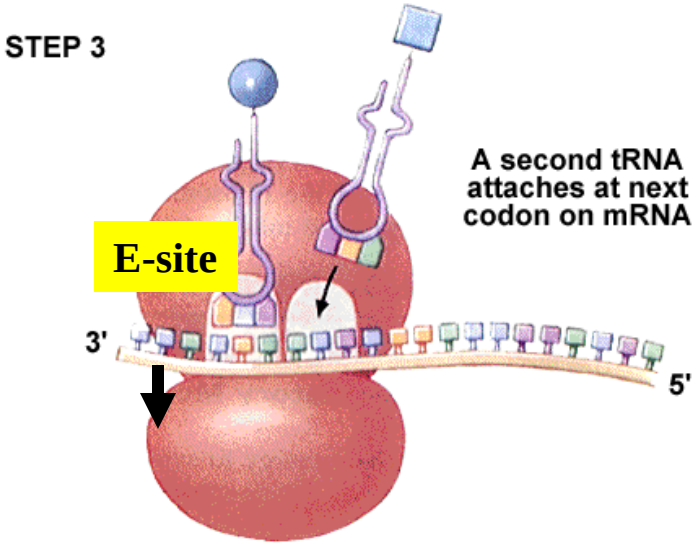


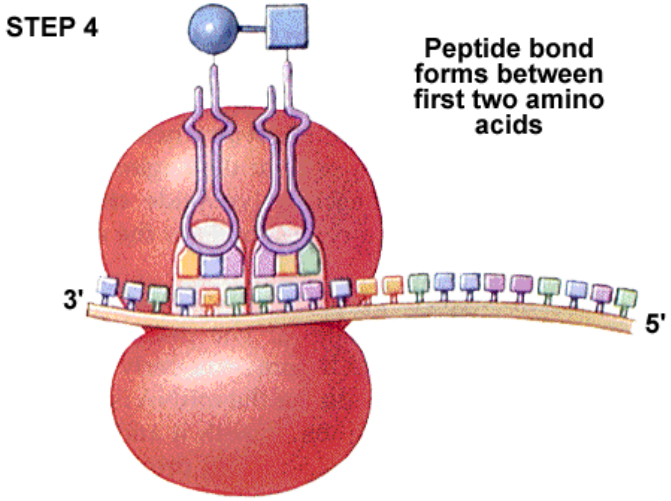
Initiation of prokaryotic protein synthesis

http://highered.mcgraw-hill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120077/micro06.swf::Protein Synthesis

Animation of prokaryotic translation, i.e., protein synthesis

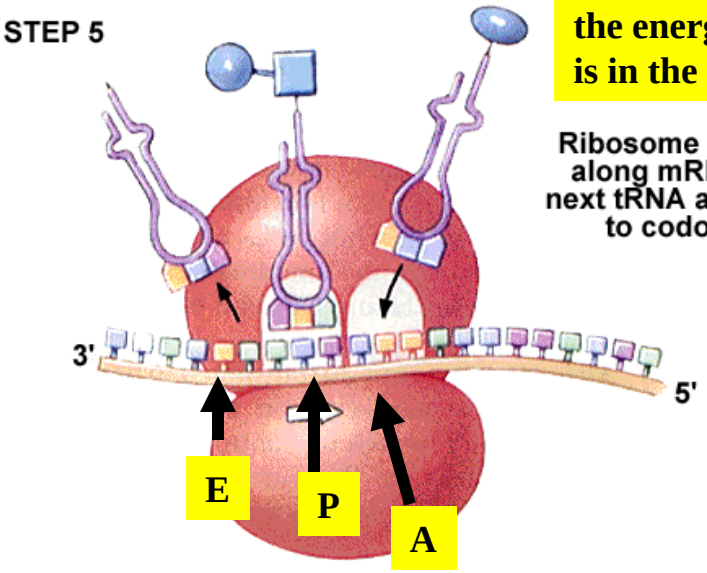
Previous slide illustrates the initiation of protein synthesis. Following the binding of the intiator codon the process proceeds as in step 3 below. The energy that drives the ribosome along the mRNA comes from GTP.



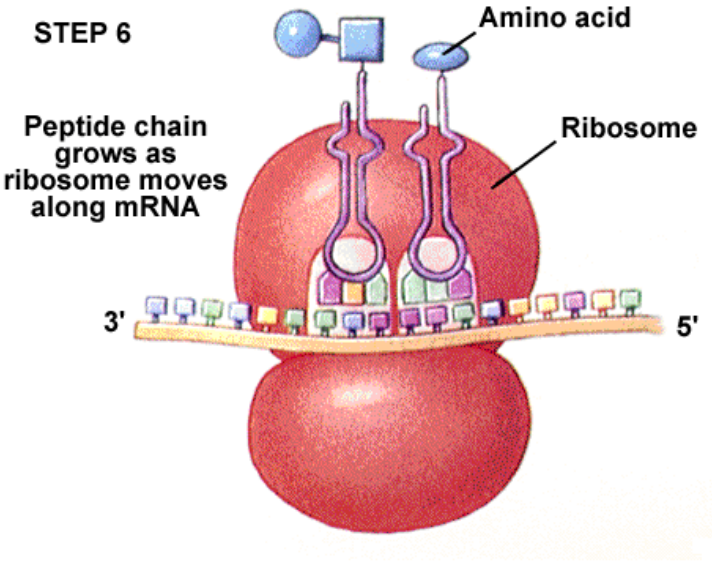


Translation: Protein synthesis uses 80-90% of the bacterial cell energy

Fig. 7.12 **Protein synthesis** – Steps 1-4



the energy to make the peptide bond is in the aminoacyl tRNA



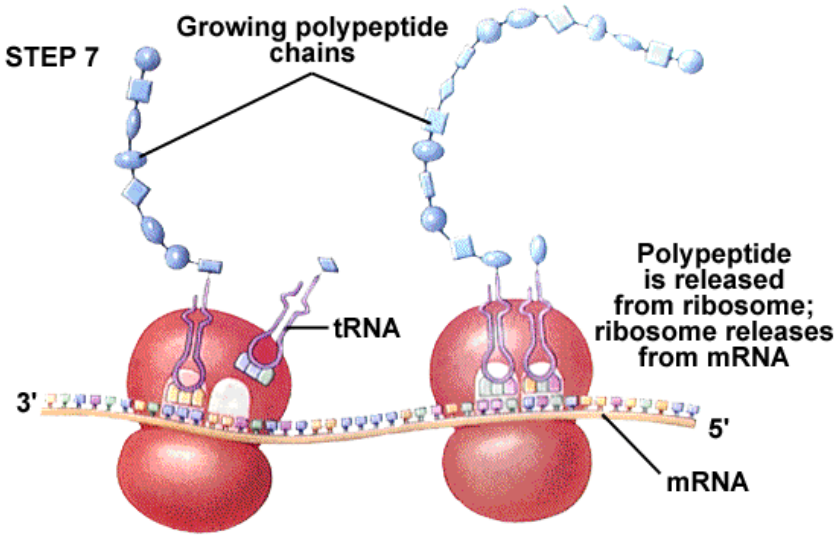


Fig. 7.14 **Protein synthesis** Steps 5-7

The Regulation of Metabolism

Categories of regulatory mechanisms

- Feedback inhibition
- enzyme induction
- enzyme repression

Feedback inhibition, also called end-product inhibition, the end product of a biosynthetic pathway directly inhibits the first enzyme in the pathway.

MS Patients Might Benefit From New Oral DrugThousands of Americans who suffer from multiple sclerosis (MS) might one day be able to take advantage of a drug that new research suggests is both safe and effective. Multiple sclerosis is a chronic and often disabling disease that affects nearly 400,000 people in the United States. It attacks the protective substance called myelin that covers the nerve fibers of the brain and spinal cord. Myelin is similar to the insulation of a wire and ensures proper nerve function. Once the myelin is damaged, the disease can also damage the nerve fibers themselves, leading to scars in these delicate tissues. This damage leads to symptoms as mild as tingling in your feet and fingers, or as severe as paralysis or blindness. Eighty-five percent of MS patients are diagnosed with what is called relapsing-remitting MS, which means they experience flare-ups of symptoms, followed by partial or complete recovery. A team of researchers looked at nearly 1,500 patients in 28 countries taking the experimental oral drug, known for now by the name BG-12, to see whether such flare-ups decreased, as well as whether they experienced any side effects from the treatment. The patients were studied for two years. The information in red is from the study on which the above article is based.

BG-12 (dimethyl fumarate) was shown to have antiinflammatory and cytoprotective properties in preclinical experiments and to result in significant reductions in disease activity on magnetic resonance imaging (MRI) in a phase 2, placebo-controlled study involving patients with relapsing–remitting multiple sclerosis.

Conclusions

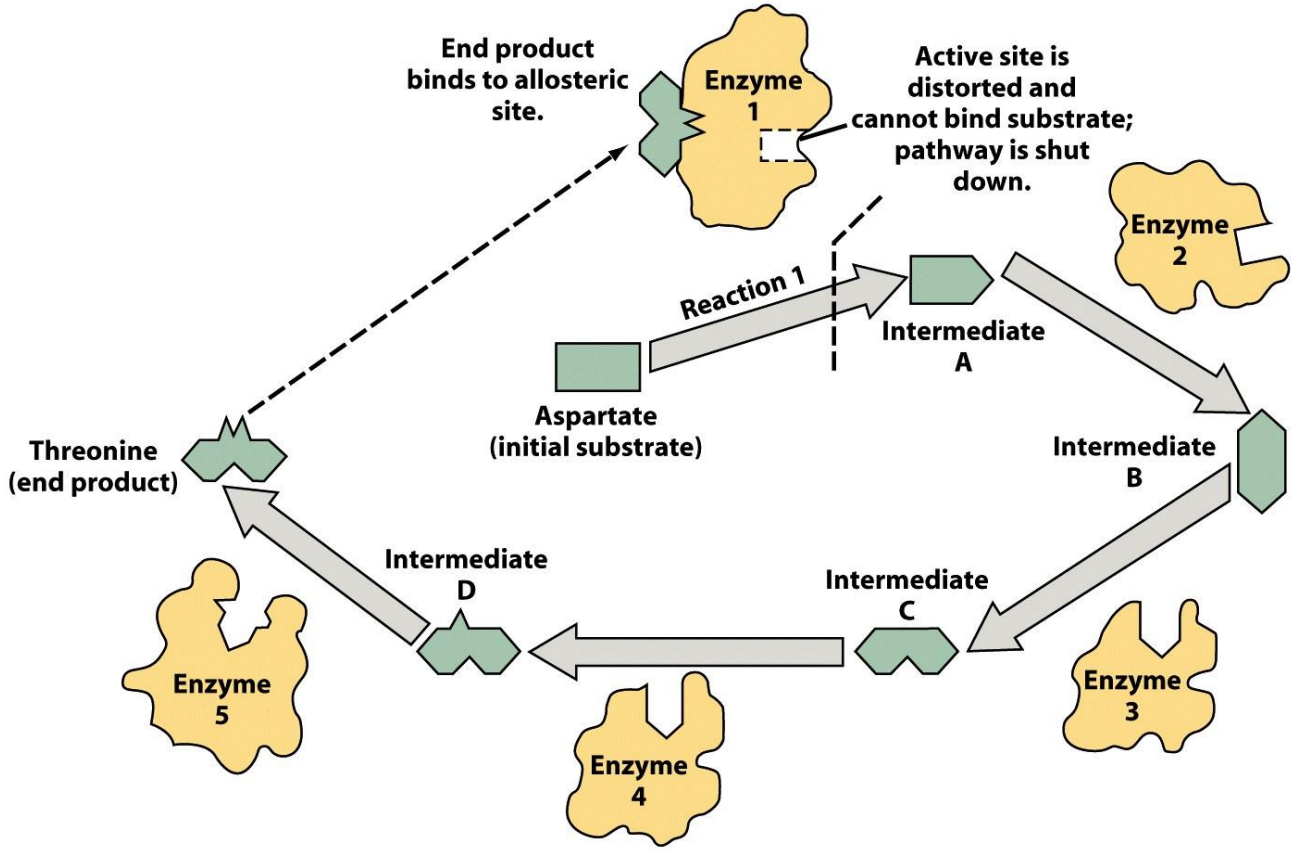
In patients with relapsing–remitting multiple sclerosis, both BG-12 regimens, as compared with placebo, significantly reduced the proportion of patients who had a relapse, the annualized relapse rate, the rate of disability progression, and the number of lesions on MRI. (Funded by Biogen Idec; DEFINE ClinicalTrials.gov number,

Study Finds Virus to Be Fast Learner on Infecting The Michigan researchers studied a virus known as lambda. It is harmless to humans, infecting only the gut bacterium Escherichia coli. Justin Meyer, a graduate student in the biology laboratory of Richard Lenski, wondered whether lambda might be able to evolve an entirely new way of getting into its host. The standard way for lambda to get into a cell is to latch onto its outer membrane, attaching to a particular kind of molecule on the surface of E. coli. It can then inject its genes and proteins into the microbe. Mr. Meyer set up an experiment in which E. coli made almost none of the molecules that the virus grabs onto. Now few of the viruses could get into the bacteria. Any mutations that allowed a virus to use a different surface molecule to get in would make it much more successful than its fellow viruses. "It would have a feast of E. coli," Dr. Lenski said. The scientists found that in just 15 days, there were viruses using a new molecule — a channel in E. coli known as OmpF. Lambda viruses had never been reported to use OmpF before. Mr. Meyer was surprised not just by how fast the change happened, but that it happened at all. "I thought it would be a wild goose chase," he said. To see if this result was just a fluke, Mr. Meyer ran his experiment again, this time with 96 separate lines. The viruses in 24 of the lines evolved to use OmpF. The researchers sequenced the genomes of the evolved viruses and were surprised to find that this transformation always required four mutations. In all the lines that could grab OmpF, those four mutations were identical, or nearly so. No single mutation could allow the viruses to start latching onto OmpF. Even three out of four mutations brought no change. Only after they developed all four mutations could the viruses make the switch.

The results suggest the mutations help the viruses do a better job of hooking onto the original molecules after they became scarce. "When you put all four together, you get this entirely new function," Mr. Meyer said. The new experiment provides a surprising glimpse at how easily viruses can evolve entirely new traits — and thus give rise to new diseases. A debate has swirled around whether a strain of avian flu called H5N1 could turn into a global killer. Last year scientists ran experiments in which a highly lethal H5N1 virus gained the ability to spread among mammals. In response to the urging of a federal advisory board, the scientists will withhold crucial details when they publish their research. But according to news reports, the team, based in the Netherlands, found that it took five mutations to transform the flu.

Regulation of Metabolism

- Feedback Inhibition (Enzymatic)



The synthesis of threonine involves five enzymatically controlled reactions (arrows) and four intermediate products (A,B, C, and D). Threonine (the end product) inhibits an allosteric enzyme (1) that catalyzes Reaction 1. The allosteric enzyme is functional when its allosteric site is not occupied and is nonfunctional when the end product of a sequence of reactions is bound to that site.

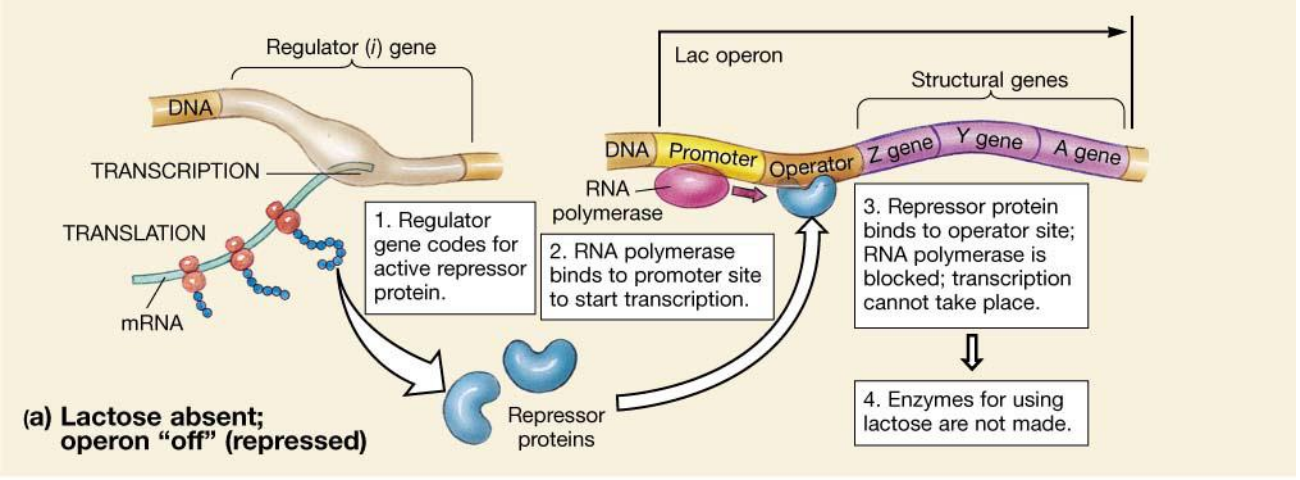
Fig. 7.13 **Feedback inhibition**

Enzyme Induction.

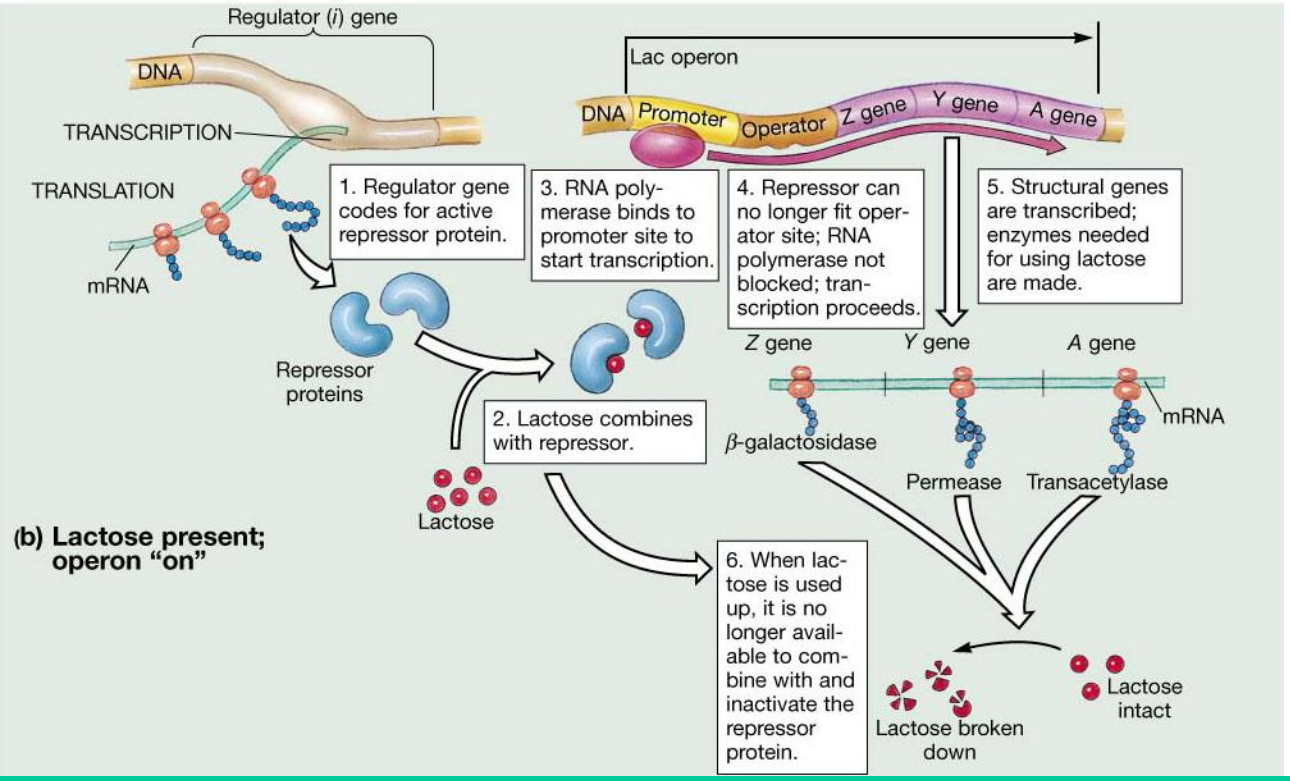
Some enzymes are maintained at comparable levels at all times in a cell. These enzymes are termed constitutive enzymes. Other enzymes are induced depending on the presence or absence of a nutrient; these are called inducible enzymes. The nutrient itself acts as an inducer of enzyme production.

Lac operon

http://www.youtube.com/watch?v=iPQZXMKZEfw&feature=related



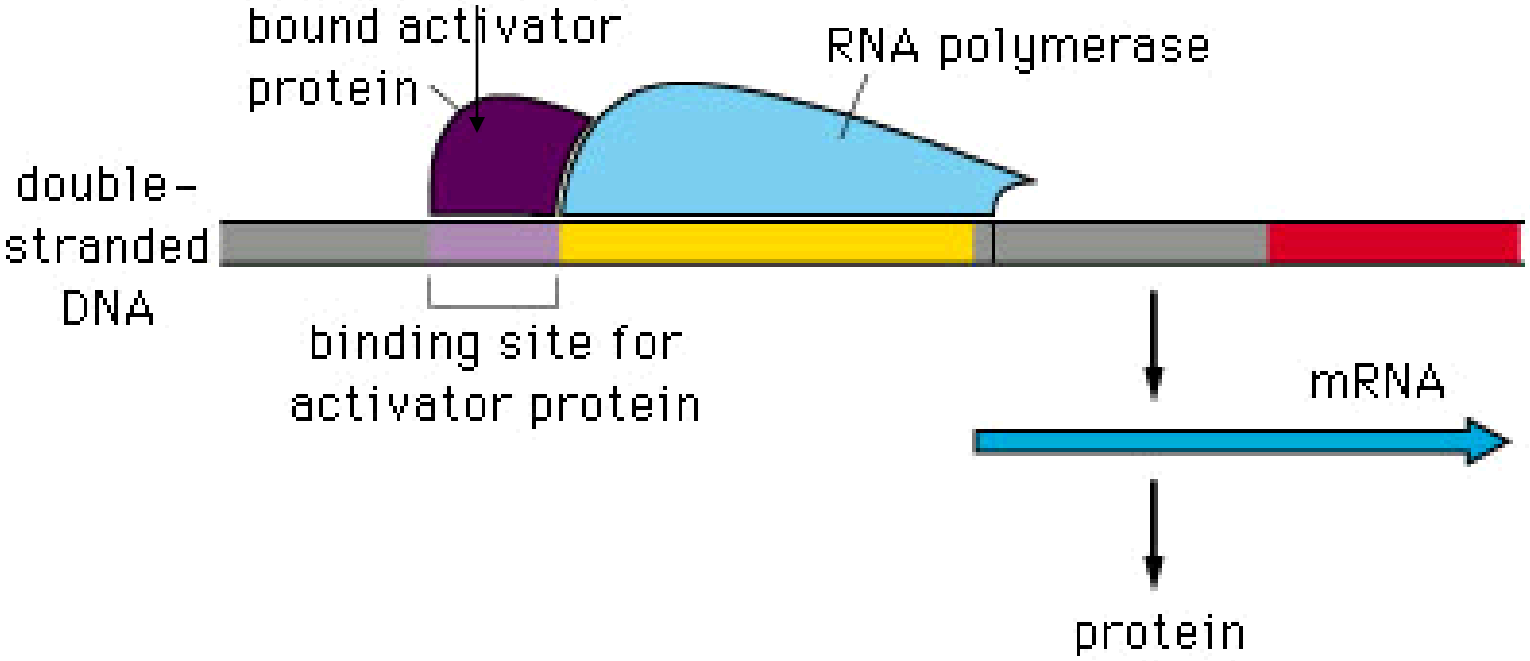
Lactose is absent the operon is "off"



Lactose is present the operon is "on"

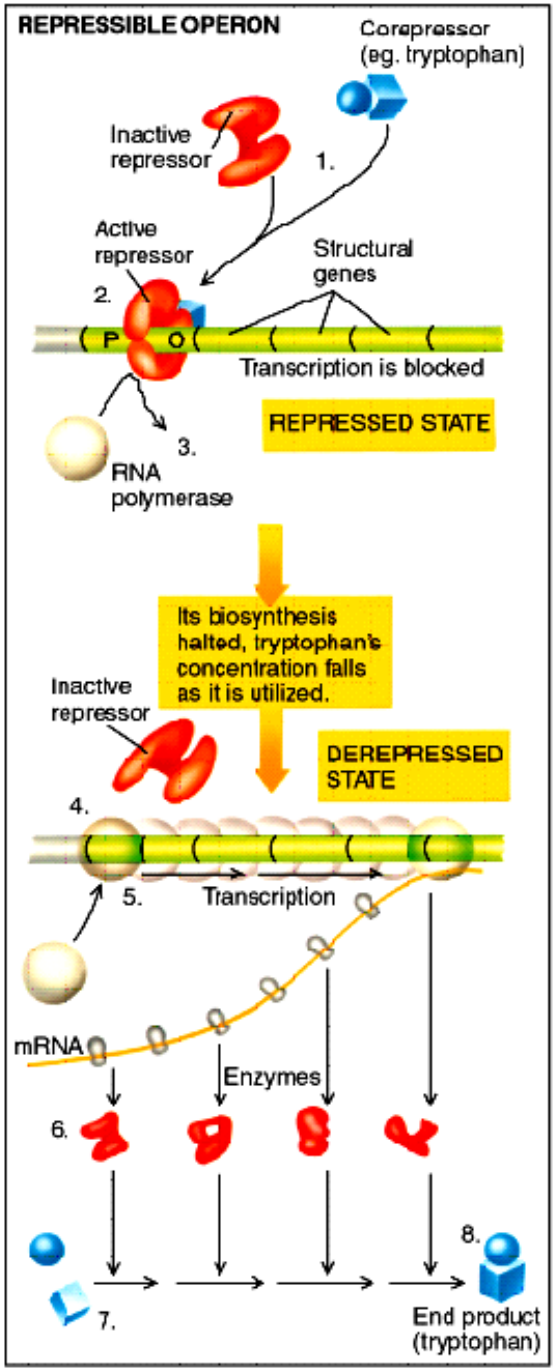
Fig. 7.14 **Enzyme induction (negative regulation because binding of the element blocks transcription)**

cAMP-CRP (cyclic AMP-cyclic AMP receptor protein)



In the presence of glucose the the *lac* operon is off because glucose decreases the level of cyclic AMP (cAMP) in the cell and cAMP is needed to activate the cAMP-CRP which is an necessary positive regulator of the *lac* operon

cAMP-CRP is an example of <u>positive regulation</u> (binding of the element is needed for the start of transcription)



Unlike what is observed in the lac operon the addition of tryptophan causes the repressor to bind and to shut off the pathway. Unlike the *lac* operon in the presence of tryptophan this regulatory system shuts off.

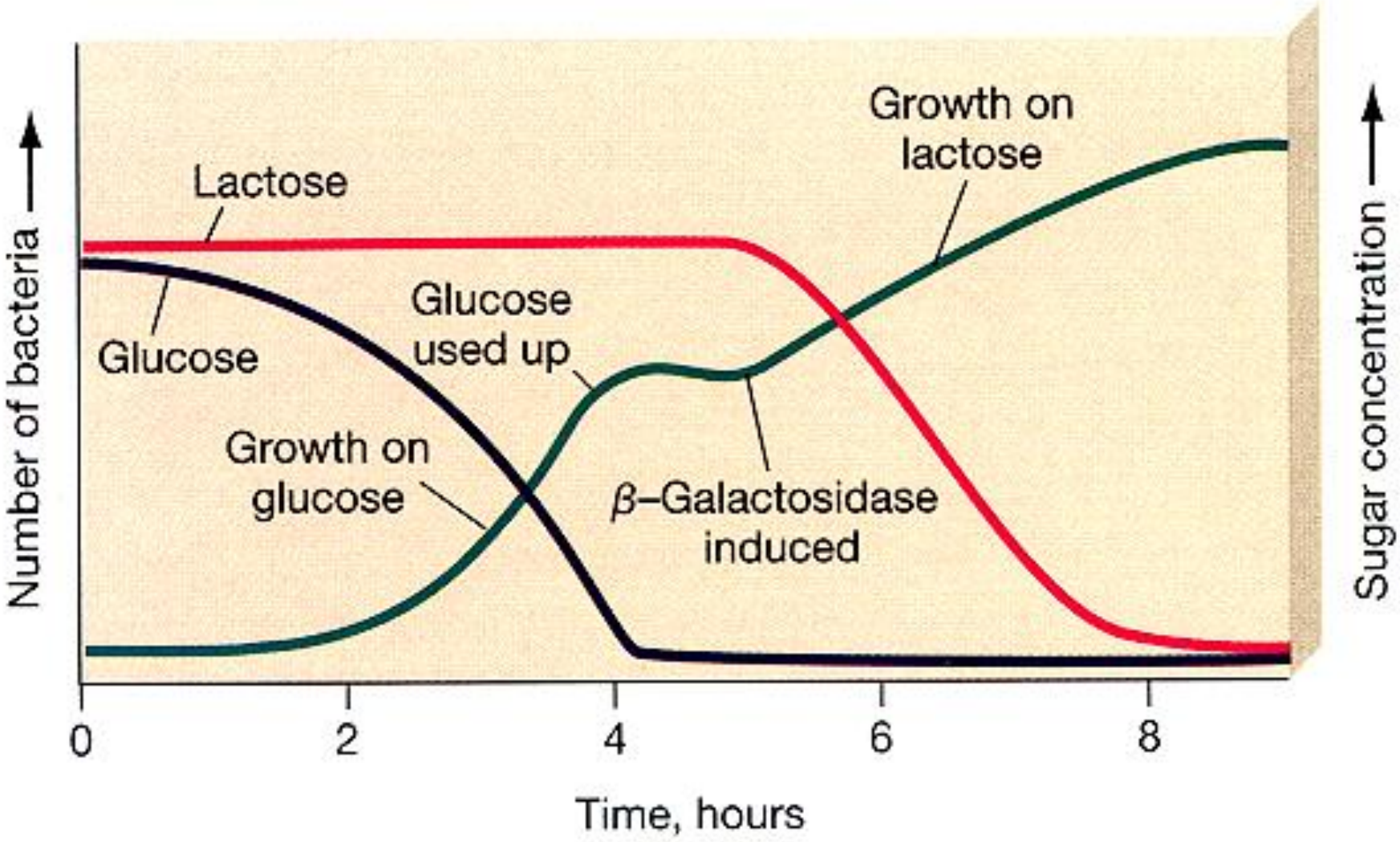
Enzyme repression

Enzyme Repression animation

http://highered.mcgraw-hill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120080/bio26.swf::The Tryptophan Repressor

Catabolite repression:

A Slightly different kind of repression operates in connection with some catabolic pathways, i.e., catabolite repression. When certain bacteria *E. coli*,, for example, are grown of a nutrient medium containing both glucose and lactose they grow at a logarithmic rate as long as glucose is available. When the glucose is depleted, they enter a stationary phase but soon begin to grow again at a logarithmic rate, though not quite as rapidly. This time the logarithmic growth rate results from the metabolism of lactose. The stationary phase is the period during which the enzymes needed to utilize lactose are induced.



The figure above shows the growth pattern of *E.coli* in medium containing both glucose and Lactose

Fig. 7.15 **Catabolite repression.** In *E. coli* grown in the presence of glucose the level of cyclic AMP is markedly reduced.

Mutations- heritable changes in the sequence of nucleotides in DNA. Mutations account for evolutionary chages in bacteria and larger organisms and for alterations that produce different strains within species.

Types of mutations and their effects.

Genotype- genetic information contained in the DNA of an organism

Phenotype- specific characteristics displayed by the organisms. Mutatations always change the genotype and the phenotype may or may not be affected.

Point mutation-involves base substitution, or nucleotide replacement, in which one base is substituted for another at a specific location in a gene.

Point mutation-involves base substitution, or nucleotide replacement, in which one base is substituted for another at a specific location in a gene.

http://www.youtube.com/watch?v=kp0esidDr-c&feature=related

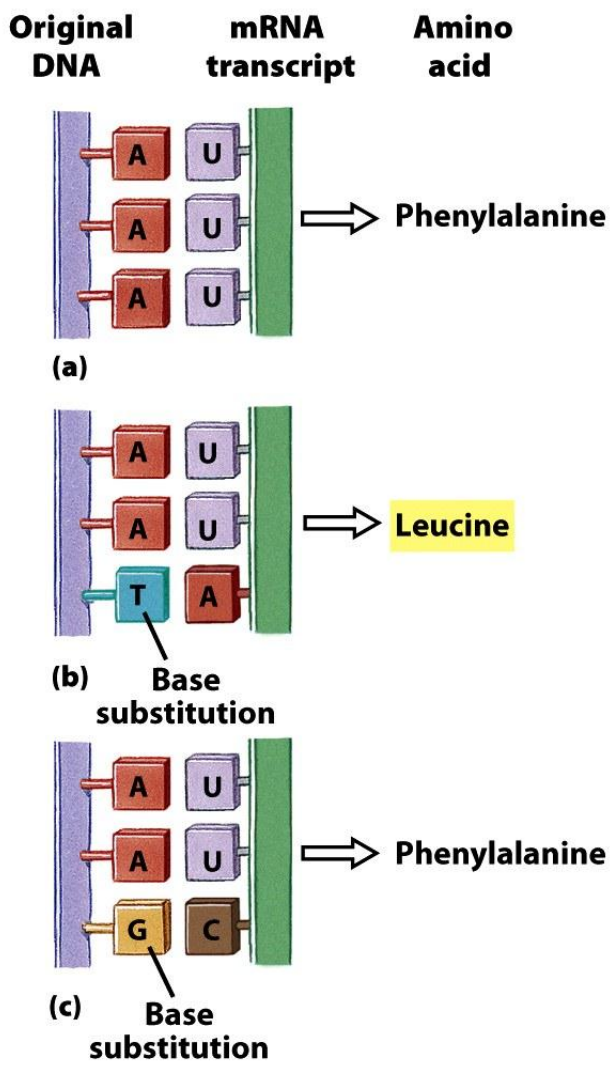


Figure 7-16 Microbiology, 7/e

http://www.youtube.com/watch?v=kp0esidDr-c

Figure 7.16- The effects of base substitution (a point mutation)

Frameshift mutation is a mutation in which there is a deletion or an insertion of one or more bases.

http://www.youtube.com/watch?v=e-xEQ05ncMo

Frameshift mutation

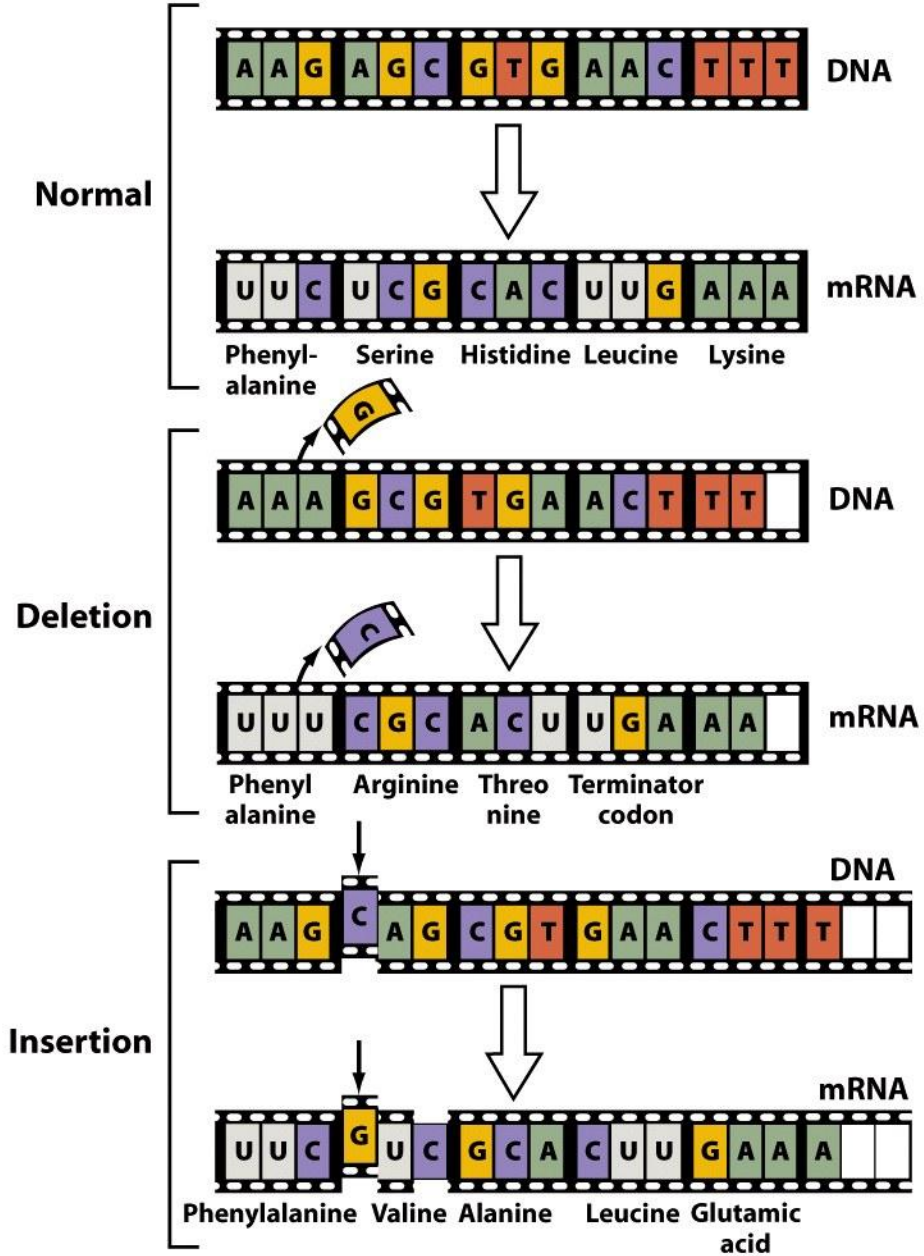


Figure 7-17 Microbiology, 7/e

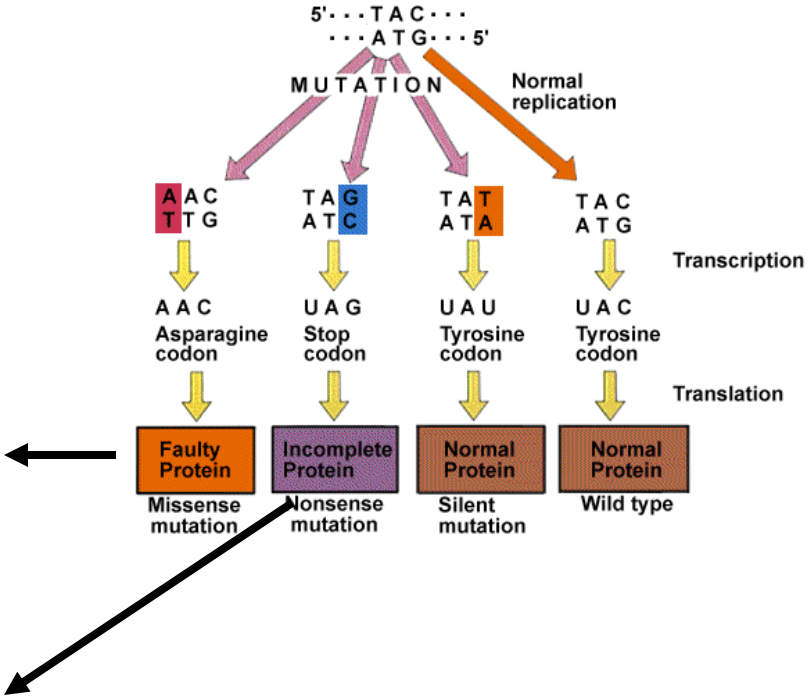
Fig. 7.17 The effects of frameshift

TABLE 7.3

Types of Mutations and Their Effects on Organisms

Types of Mutations	Effects on Organisms
Point Mutation	
Single base change in DNA with no change in the amino acid specified by the mRNA codon.	No effect on protein; a "silent" mutation.
Change in DNA with change in the amino acid sequence specified by the mRNA codon.	Change in protein by substitution of one amino acid for another; can significantly alter function of protein.
Change in DNA that creates a terminator codon in mRNA.	Produces polypeptide of no use to organism and prevents synthesis of normal protein.
Frameshift Mutation	
Deletion or insertion of one or more bases in DNA.	Changes entire sequence of codons and greatly alters amino acid sequence; can introduce terminator codon and produce useless polypeptides instead of normal proteins.

Table 7-3 Microbiology, 6/e



Type of mutations and their Effects on organisms

http://www.youtube.com/watch?v=y-8OQoh9_FM&feature=related

Missense mutation

Auxotrophs: require a special nutrient to maintain growth; Prototrophs: wild type

Phenotypic variation- Studies of bacteria that have lost the ability to synthesize a particular enzyme have played an important role in our understanding of metabolic pathways.

Such nutritionally deficient mutants are called **auxotrophs.** Auxotrophs require a special nutrient to maintain growth. For example an arginine auxotroph of *E. coli*, requires arginine for growth,

In contrast, the **Prototrophs , or wild type of of** *E.coli*, makes its own arginine.

Another type of phenotypic variation of genetic origin is **temperature sensitivity**.

For example due to a point mutation in a critical nutrient an organism may loose the ability to grow at 40c but grows fine at 37C.

This phenomenon may be due to a point mutation that changed a single amino acid in an enzyme thereby making the enzyme (protein) heat sensitive.

Thus, one can study characteristics of the organism at 37C, that is conditions under which the protein functions perfectly well, and then shift the organism to 40C, shut off the protein, and determine the effects on growth of loss of this thermolabile protein.

AIDS science leaping ahead, but will the money follow? CHICAGO (Reuters) - Last year, the HIV/AIDS community got some startling news. Lifesaving drugs known as antiretrovirals that have brought millions of AIDS sufferers back from the brink also dramatically cut the risk that they will transmit the virus to their loved ones - by as much as 96 percent. The landmark study, known as the HIV Prevention Trials Network 052 trial, proved that AIDS treatment was also a powerful form of prevention. Science magazine dubbed it the 2011 Breakthrough of the Year. The findings - along with studies on the preventive benefits of circumcision and treating high-risk individuals before they are exposed to HIV - have been heralded as weapons that could finally break the back of the AIDS epidemic. But fully rolling out treatment as prevention would mean more than doubling current HIV treatment goals, from the current United Nations target of treating 15 million by 2015 to 34 million, a staggering increase. "The benefits of early detection and treatment have never been more clear, but countries have never been more challenged to provide needed resources," Kaiser Family Foundation Chief Drew Altman said in a statement

Autism Gastro Problems May Be Linked to Gut Bacteria -- Children with autism have bacteria in their gut that is different from the bacteria seen in kids who do not have the disorder, researchers have found. In their report, published Jan. 10 in the online journal *mBio*, researchers from the Mailman School of Public Health at Columbia University in New York City suggested that this finding could help explain the link between autism and gastrointestinal problems, such as inflammation. The relationship between different microorganisms and the host and the outcomes for disease and development is an exciting issue," the study's editor, Christine Biron, a professor of medical science at Brown University, said in an American Society for Microbiology news release. "This paper is important because it starts to advance the question of how the resident microbes interact with a disorder that is poorly understood."The researchers found a relatively large amount of *Sutterella* bacteria in 12 out of 23 tissue samples taken from the guts of children with autism. In contrast, they did not find this type of bacteria in any samples taken from children without autism who were studied for comparison. "*Sutterella* has been associated with gastrointestinal diseases below the diaphragm, and whether it's a pathogen or not is still not clear," explained a reviewer of the research, Jorge Benach, chairman of the microbiology department at Stony Brook University. "It is not a very well-known bacterium," he pointed out in the news release. The findings are significant because digestive complications can be very serious in kids with autism, contributing to their behavioral problems, the study authors noted.

The study results are also more definitive than previous studies that used stool samples, because tissue samples surgically removed from the gut are more reflective of the bacteria found in the children's intestinal walls.

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Spontaneous and induced mutations

Spontaneous mutations occur in the absence of any agent known to cause changes in DNA- often due to errors in base pairing of nucleotides in the old and new strands of DNA and range from 1 in 10^{-3} to 1 in 10^{-9} per cell (average around 1 in 10^{-6} per cell).

Induced mutations are produce by agents called mutagens, which increase the mutation rate above the spontaneous mutation rate.

http://www.youtube.com/watch?v=HYS6EKnQcv0&feature=related

Repair of point mutations

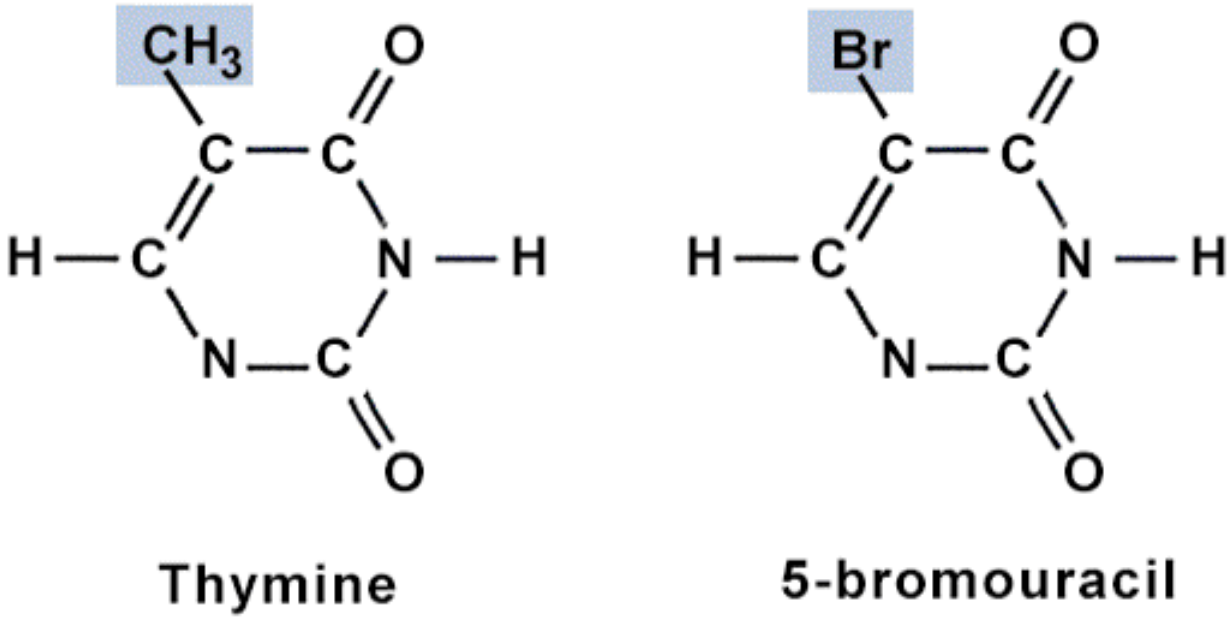


Fig. 7.18 **Base analogs**

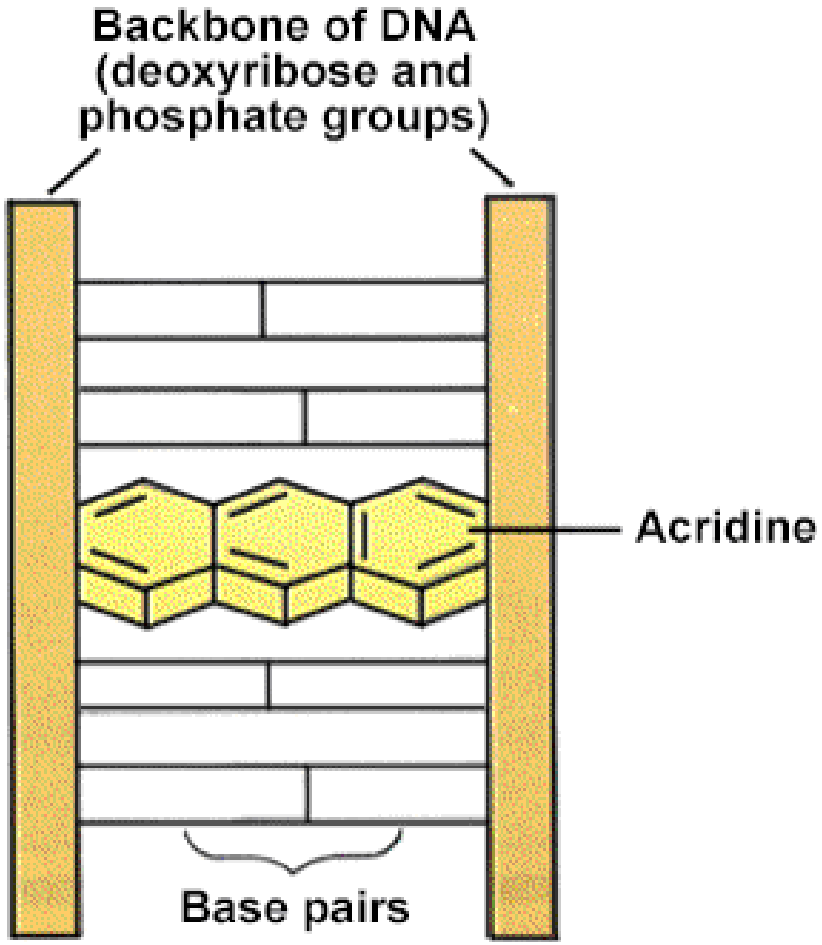


Fig. 7.19 **Acridine, a chemical mutagen.** Insertion of acridine orange Into DNA helix can produce a frameshift

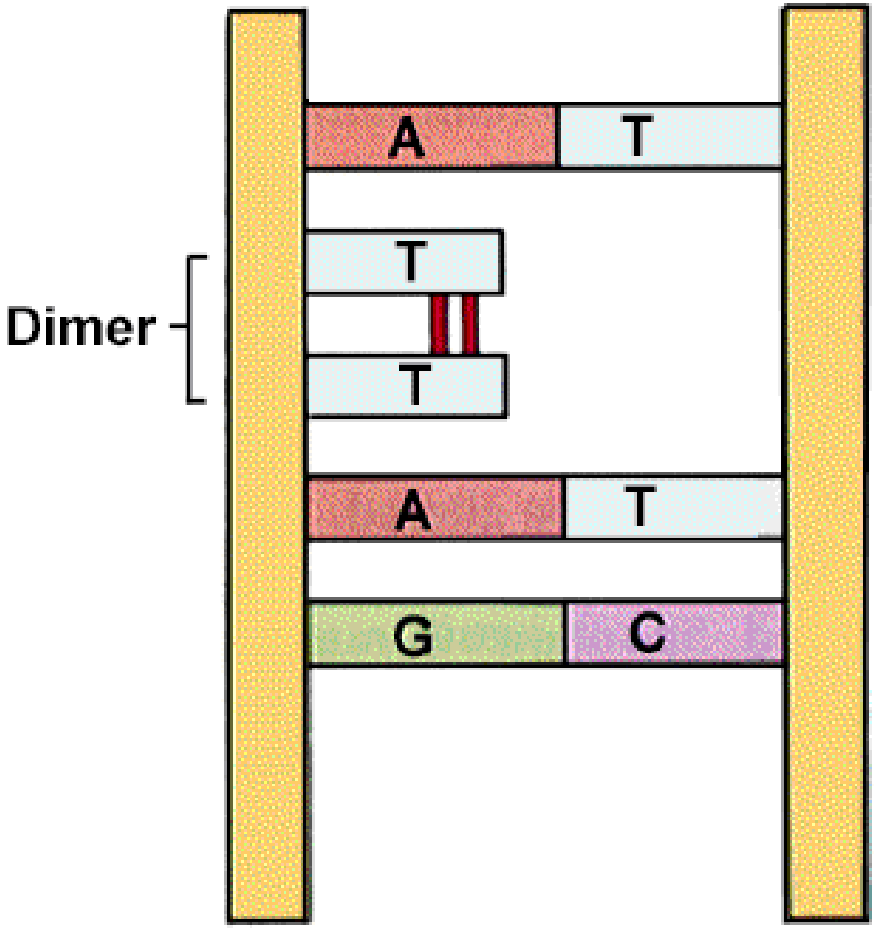


Fig. 7.20 **Thymine dimers caused by radiation**

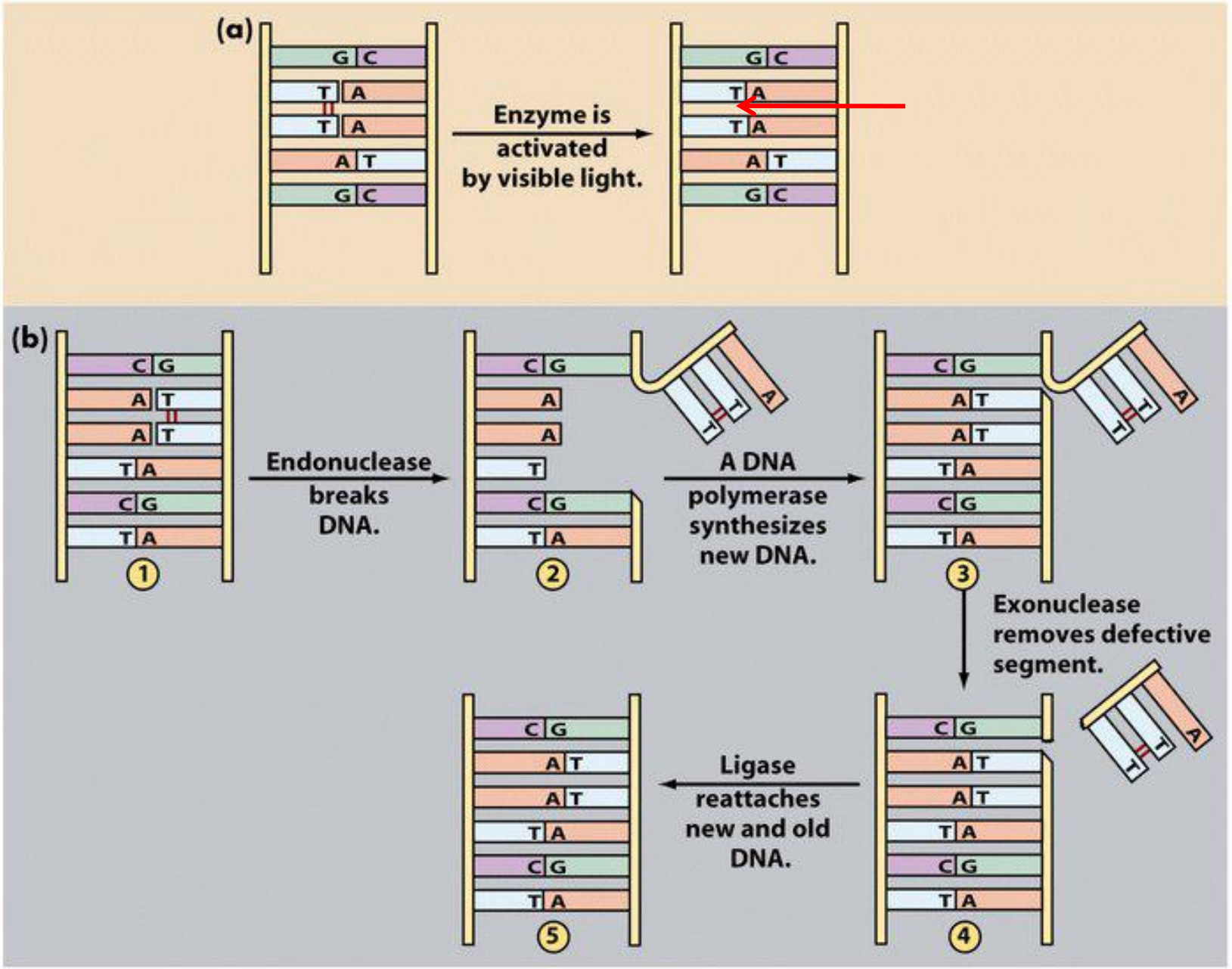
Thymine dimers: formation and repair

http://highered.mcgrawhill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120082/micro18.swf::Thymine Dimers

The repair of DNA damage

Light repair or photoreactivation , occurs in the presence of visible light in bacteria previously exposed to UV light.

Dark repair, a defective segment of DNA is cut out and replaced. Some human skin cancers, such as xeroderma pigmentosum are caused by a defect in the cellular DNA repair mechanism



Light

Dark

http://highered.mcgrawhill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120082/micro18.swf::Thymine Dimers

Figure 7.21 Thymine dimer reparis

repeat of the thymine dimer slide

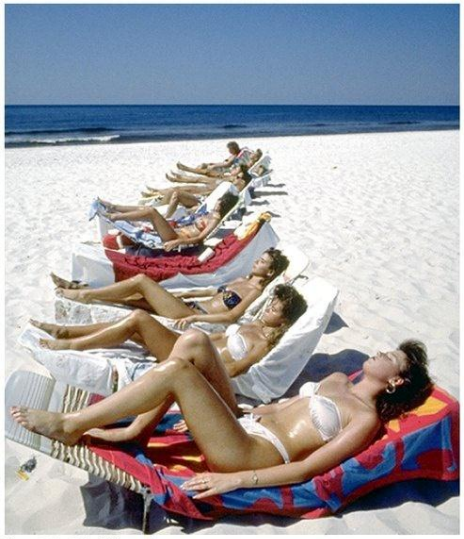


Figure 7-22a Microbiology, 6/e
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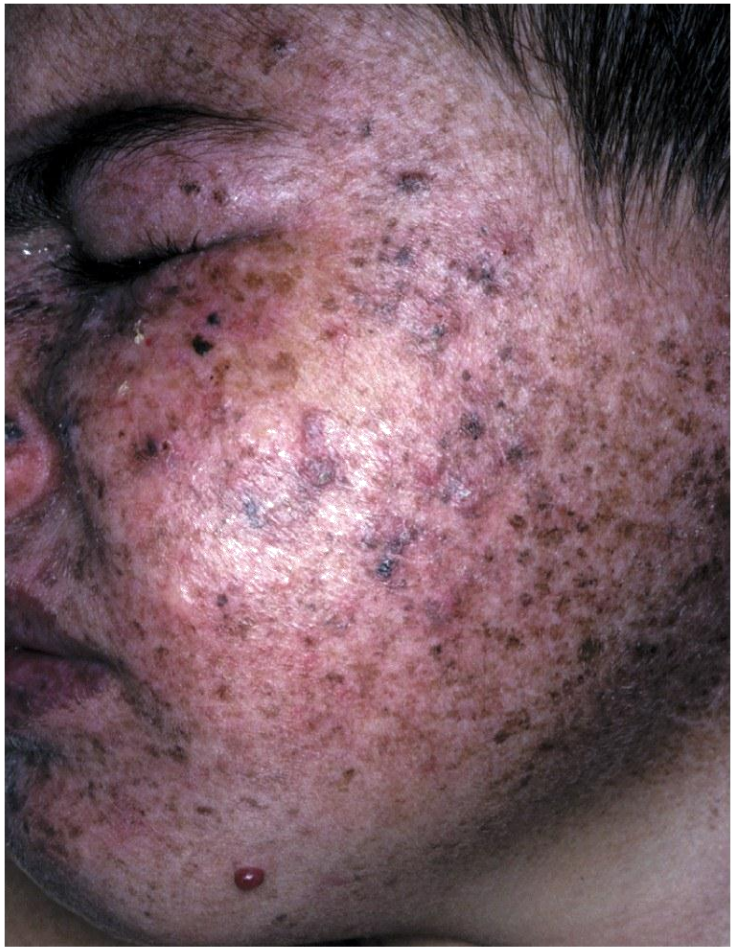


ISM/Phototake

Fig. 7.22 **The inability to repair UV-caused dimers**

Hypothesis: a tentative explanation for an observed condition or event.

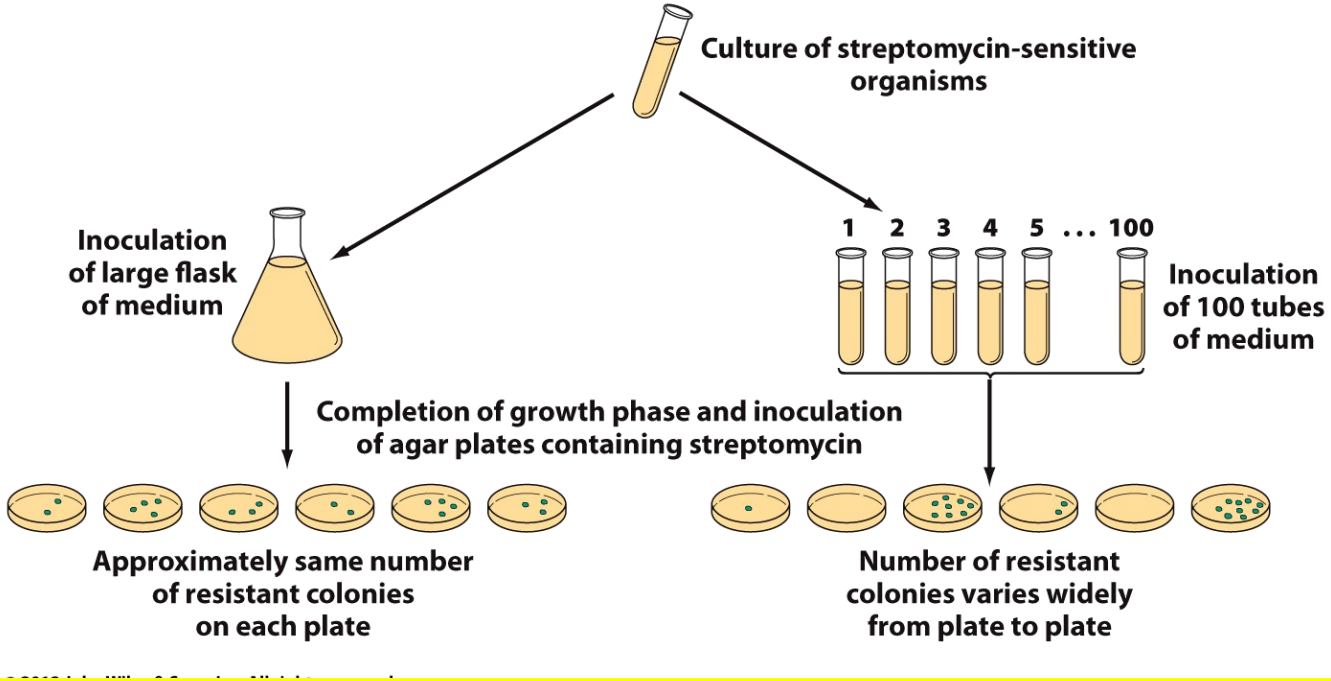
Fluctuation test (next slide) is based on the following possible hypotheses:

1) If mutations that confer resistance occur spontaneously and at random, we would expect great fluctuation in the number of resistant organisms per culture among a large number of cultures.

2) If on the other hand, mutations occur as a result of exposure to the drug then we would expect similar numbers of resistant organisms per culture among a large number of cultures.

Fluctuation Test

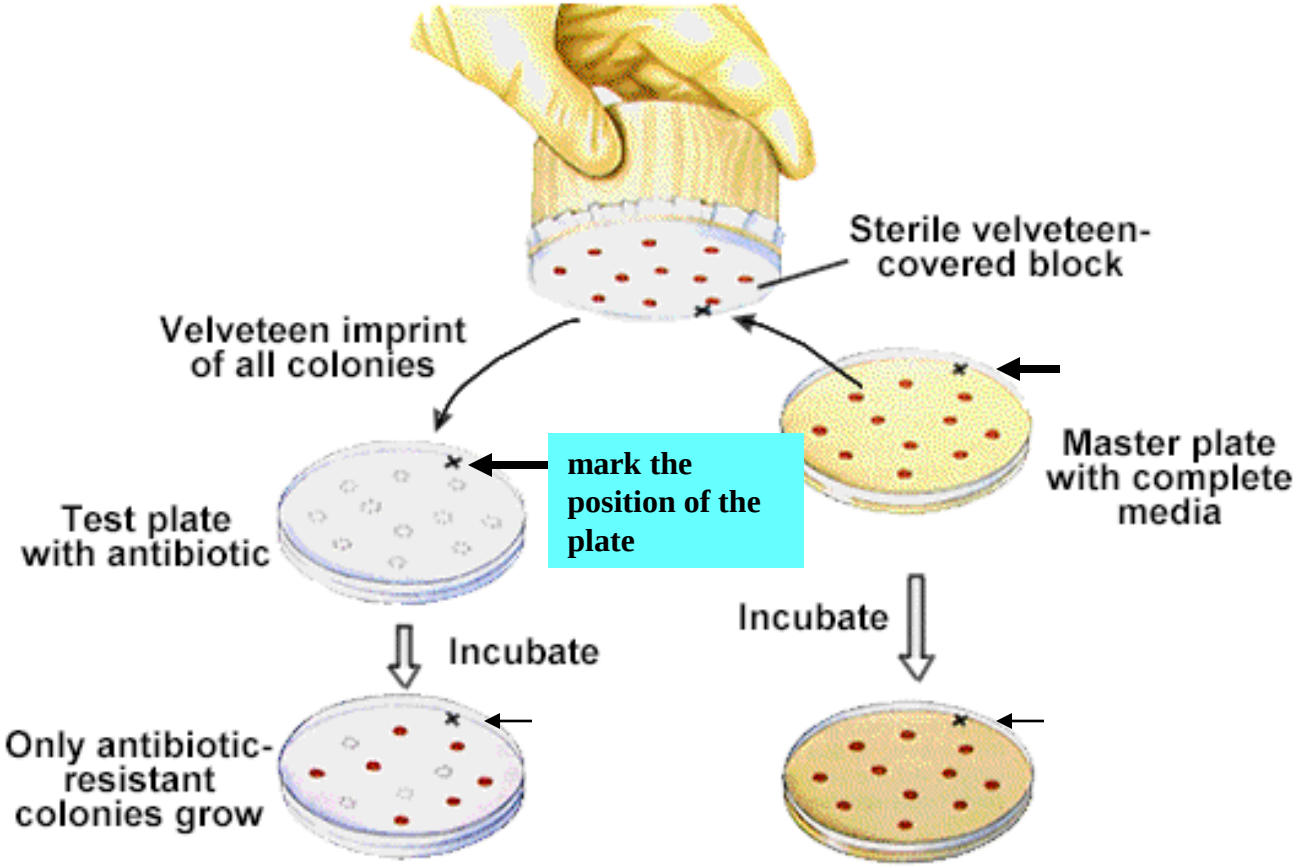
- Luria/ Delbruck
- Mutation occurs randomly and spontaneously
- Supporting Evolutionary theory



Luria and Delbruck's fluctuation test supports the hypothesis that mutations conferring antibiotic resistance are random and are not produced by exposure to the antibiotic. This conclusion is based on the observation that there is significant variation in the number of resistant colonies on the antibiotic containing plates.

Fig. 7.23 **Fluctuation testing-**

Isolating particular mutants from a culture containing both mutated and normal organisms.



This technique allows detection of antibiotic-resistant organisms. The X on the side of the plate provides a reference for identifying colonies from the same organism.

Fig. 7.24 **Replica plating**

Ames test – used to determine whether a particular substance is mutagenic, based on its ability to induce mutations in auxotrophic Bacteria- This test is the first line test in commercial products for determining whether or not a product is potentially carcinogenic- despite the fact that the Ames test does not test for carcinogenicity.

Principle: Gain of function mutation is used in this test, i.e., the acquired ability to make histidine if the cell is mutated.

Very important first-line test for all products that come into human contact. Second line is typically tissue culture followed by animal testing (a number of so-called tumor promoters are negative in the AMES test because they are NOT mutatgens, but are capable of causing cancer in animals-tumor promoters typically stimulate signal pathways, such as PKC, which MAY transform a benign tumors into a malignant one or even initate uncontrolled cellular growth). These agents are often tested on the animal skin.

Ames Test

- **Detects mutagens- (not carcinogens)**
- ***S. typhimurium* (his$^-$)**
- **Rat Liver Extract**

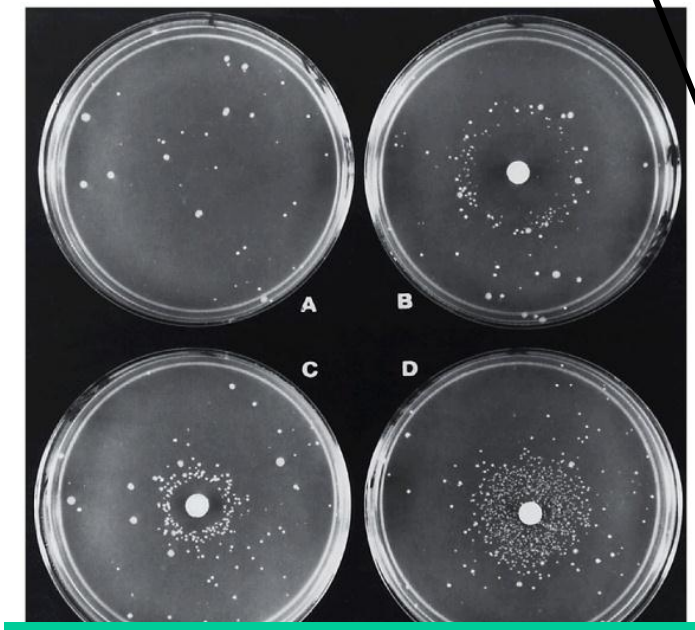
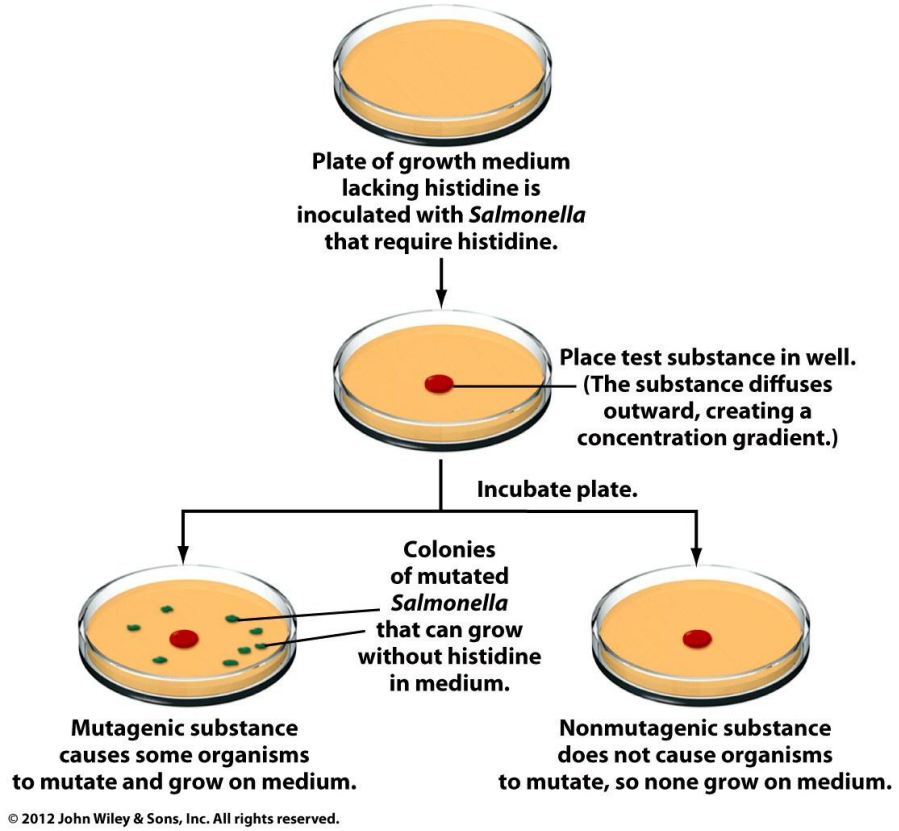
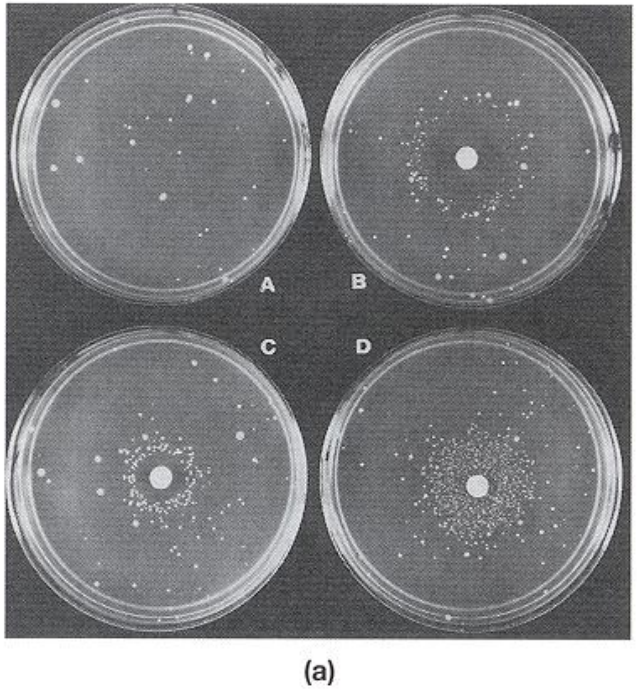


Figure 7.25 AMES TEST

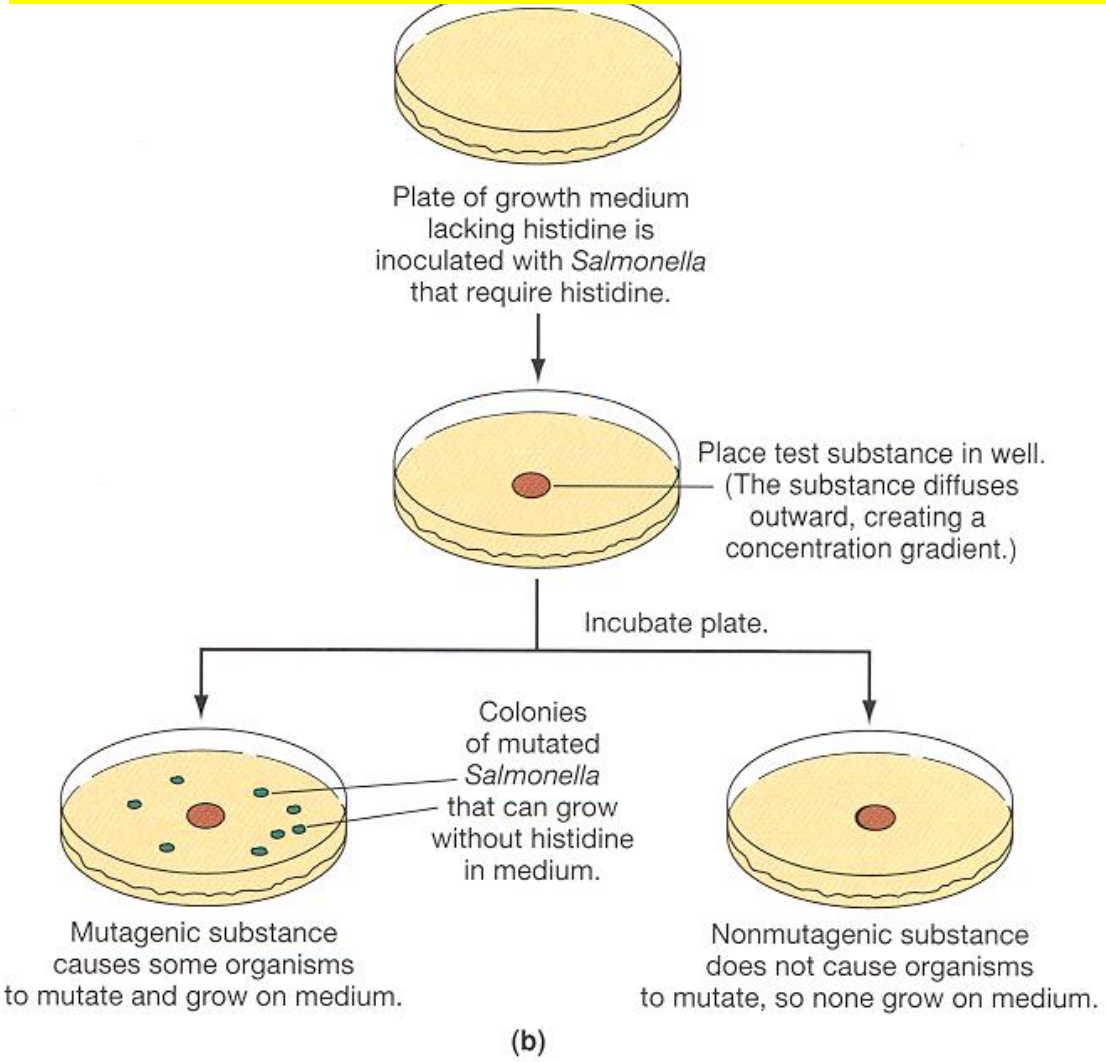


Mix test substance with <u>microsomal fraction</u> from liver to activate Procarcinogens- before plating the test substance- important step because bacteria lack an endoplasmic reticulum which is needed to convert many procarcinogens into mutagens

Ames tests looks for a gain-of-function (i.e., the ability to make histidine, following a mutation, in cells that are histidine auxotrophs)



(a)

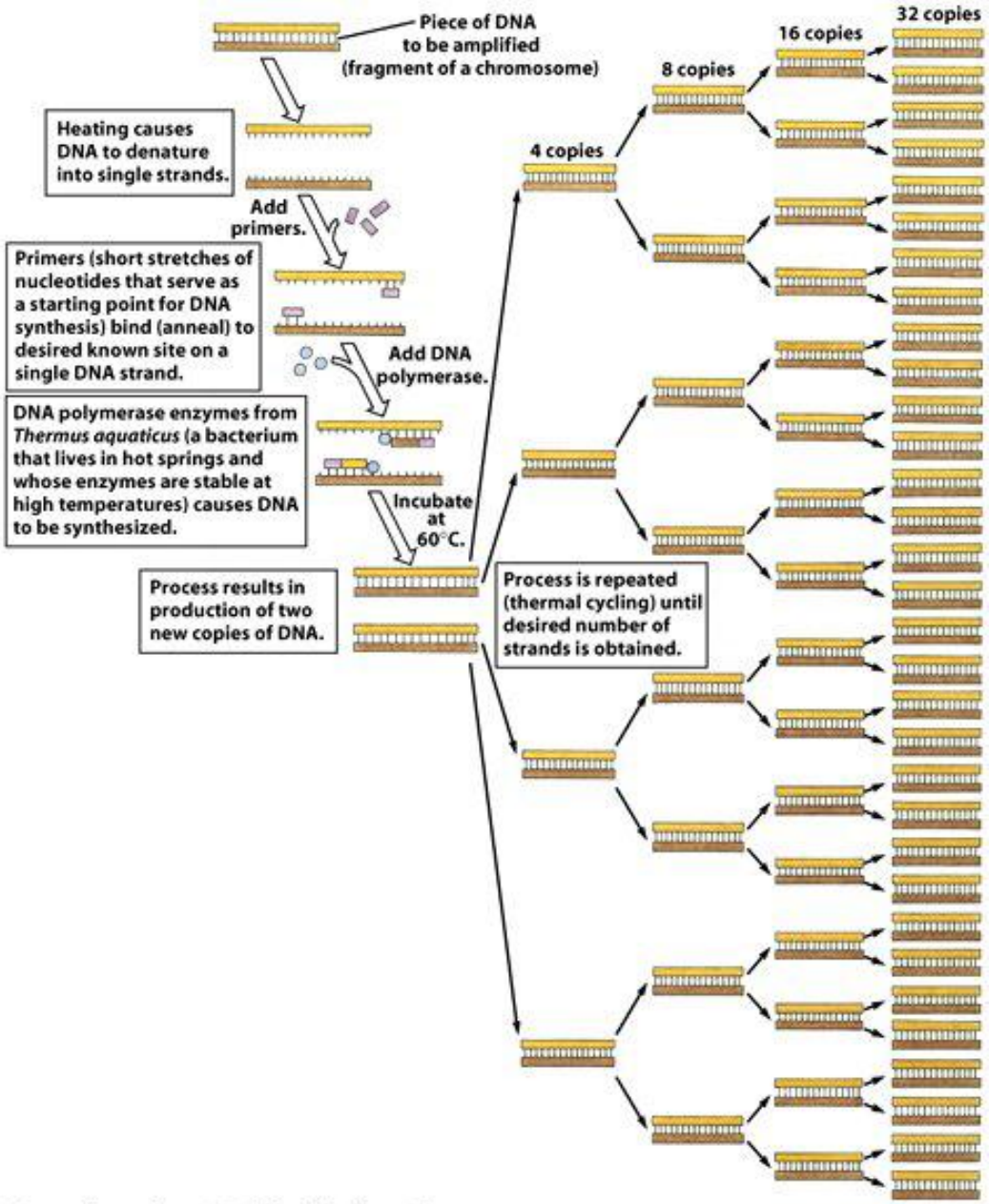


(b)

est. (b) The test is used to determine whether a
rcinogen.

Mix test substance with microsomal fraction from liver to activate Procarcinogens- before plating the test substance- important step because bacteria lack an endoplasmic reticulum which is needed to convert many procarcinogens into mutagens

Figure 7.25 AMES TEST



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Polymerase chain reaction (PCR)

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