- consists of a distinct n ______ with nuclear membrane in eucaryotes
- size varies, 1_____ μ in length average packed into a cell only 1 to 2 μ (like a ball of string)
- DNA replicates f ______ than cell divides cells will have more than one copy on average
- high n _____ charge, requires positive ions to neutralize the charge (Mg⁺⁺, Ca⁺⁺, and polyamines) to wrap up in a ball

Nucleic Acids

- deoxyribonucleic acid (DNA) consists of several million b _____ p ____ (nucleotides): adenine, guanine, cytosine, and thymine. Guanine pairs with cytosine on the c ______ strand, and adenine pairs with thymine. Bonding is through h ______-bonds.
- 5-carbon sugar (deoxyribose), p group, and n containing group base linked to #5 and #1 carbons
- in ribonucleic acid (RNA) u_____ replaces thymine and the 5-carbon backbone is ribose instead of deoxyribose (contains an OH group on the #2 carbon)

DNA Replication

- b_____ strands of DNA (sense and anti-sense) are copied at the same time
- r_____ of replication is always constant (i.e., independent of growth)
- can't initiate fork in d______ strand until replication of parent strand is finished
- initiation of new f______ increases as growth rate increases
- at least 20 different p_____ and enzymes required for DNA replication (e.g. DNA polymerase)
- 7 steps:
 - 1. Recognition of o_____ for replication
 - 2. U_____ of DNA strands
 - 3. Holding apart of DNA t______ strands
 - 4. I_____ of new daughter strand
 - 5. E_____ of daughter strands
 - 6. R_____ of daughter strands
 - 7. T______ of replication

Transcription

- process of creating m______ RNA (mRNA) from a segment of the DNA b______ based on start (promoter) and stop signals
- s_____ of DNA encoded for enzymes for a sequential series of reactions is called an operon
- mRNA is the "s_____ copy" of DNA blueprint
- a single mRNA usually contains i ______ for producing a number of related enzymes or may be for a single enzyme
- catalyzed by RNA p_____
- mRNA is u_____, degrades 2minutes after synthesis (conserves resources)
- enzyme r_____ and I_____ occurs at the level of transcription

Translation

- mRNA contains information for the sequence of a ______a that make up a protein molecule (e ______ are proteins, protein structure and function depend solely on amino acid sequence)
- each 3 sequential bases (called a c_____) specify a particular amino acid, also have codons for start and stop signals for each protein
- transfer RNA (tRNA) will transfer a particular amino acid to the mRNA,
- tRNA is smallest of the three types of RNA and is not specific to a particular enzyme, but is particular to an amino acid
- tRNA has a complementary set of bases called an a _______ specific for the codon on the mRNA
- amino acids are attached to tRNA, requires e_____ in the form of ATP
- assembly of proteins occurs on the r _____ (or rRNA), rRNA is the w _____ for protein assemble and constitutes approximately 80-90% of RNA in a cell
- assemblage of proteins occurs rapidly with about amino acids added per second
- rRNA is not specific to a particular e______

Plasmids

- a_____, self replicating, extrachromosomal, double stranded, circular DNA. Vary in size from 10 to 1000 kbp.
- *c______ plasmids* carry genes that code for their transfer to other cells
- *resistance t______ factors* (factors) are plasmids that confer resistance to antibiotics
- Col factors are plasmids that code for c_____
- *catabolic plasmids* code for e______ for degradation of specific xenobiotic compounds (e.g., naphthalene, toluene, salicylate)
 - 1. Nomenclature:
 - copy number low (1-2 copies per cell) high (10-100 cpc)
 - stringency relaxed (do not require replication for amplification) versus stringent (requires replication, therefore not amplified)
 - incompatibility depends on their ability to coexist within the same cell

Genetic Recombinations

Transformation:

- e_____ DNA enters competent recipient
- DNA f ______ splits into two single strands: one strand is integrated into r ______ DNA, other strand is degraded

Conjugation:

• genetic material (plasmid or DNA fragment mobilized by plasmid) is t______ from cell to cell by sex pilus during conjugation

Transduction:

• genetic material is t______ through a bacterial phage (bacteriophage is a virus that attacks bacteria, see Figure 1.22)

Transposition:

- plasmid or chromosomal DNA changes p_____ (i.e., jumps) from one location on the genome to another
- Genetic Engineering
 - in v_____ (changes to genome in living cells) or in v_____ (changes to genome in test tube)

Steps involved:

- I_____ of source DNA
- DNA f_____
- DNA 1
- Incorporation of recombinant DNA into a h_____
- Selection of successful c_____

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Application of GEMs
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- biodegradation of x_____(e.g., dioxin)
- bioremediation
 - isolates of *Pseudomonas* that can grow in 50% t______
- biosensors
 - *l*_____ gene codes for luminescence: when biodegradation is occurring culture emits light and luminescence is proportional to degree of d______ (Gary Saylor's group)
- others???

Probe technology

- methods to i_____ and q_____ specific microorganisms in environmental samples
 - c_____ based methods
 - e_____ microscopy (TEM, SEM)
 - a_____ probes
 - g_____ probes
 - 1.often are sfor 16S rRNA
 - 2. will bind to complementary sequence on t_____
 - 3. require a m_____ for identification (fluorescence, radiolabel, etc.)
- environmental applications:
 - 1. detection of p_____
 - 2. detection of specific g_____ in samples (e.g., metal resistance, antibiotic resistance, degradative enzymes)
 - 3. d_____ and enumeration of specific bacteria
 - 4. determination of microbial community s______ to optimize operational performance of engineered wastewater treatment system

Bacteria Types

Typical

- c_____(spherical, e.g., *Streptococcus*)
- b_____ (rod shapes, e.g., *Bacillus*
- subtilis)
- s_____ (spiral, e.g., *Spirillum volutans*)
- f_____

Unusual

- s_____ bacteria filamentous, surrounded by a sheath
- s_____ bacteria aerobic, gram negative, at end of stalk is a "holdfast" allows it to attach to surfaces



Figure 3.20 Examples of bacterial cell morphology (adapted from Tchobanoglous and Schroeder, 1987)

commas or incomplete spirals. The smallest bacteria (of the genus Mycoplasma) are 0.1 to 0.2 μ m in diameter, while the mid-size is about 1.1 to 1.5 μ m in diameter (Escherichia coli) with the longest up to 500 μ m.

- b_____ bacteria, multiply by budding, bud grows flagellum, settles on new surface and buds again
- <u>g</u>_____ bacteria, filamentous, gram-negative, "glide" along solids surfaces, *Beggiatoa* and *Thiothrix*: oxidize H₂S to S⁰
- *Bdellovibrio* s_____(0.2-0.3µ) flagellated bacteria that prey on gram-negative bacteria
- Actinomycetes gram-positive, f_____, have branching filaments similar to fungi *Streptomyces* and *Nocardia*

Fungi

- e_____, produce long filaments called hyphae containing c_____
- heterotrophs, use o _____ compounds for carbon and energy
- found during n_____ limitations, low D.O., low pH conditions
- important in the cycling of organics degradation of plant polymers cellulose and lignin
- primarily aerobic (except for fermentative yeast)



FIGURE 2.18 Structure of a typical algal cell.

Algae

- most are u_____, floating, phytoplankton
- some are f_____
- most are p_____
- all contain c______a, some b and c
- found in o_____ ponds, polishing ponds, aerobic lagoons

Protozoa

- unicellular
- heterotrophs
- classification
 - sarcodina (amoebae)
 - mastigophora (flagellates)
 - ciliophora (ciliates)
 - sporozoa

Viruses

- small c_____ particles (not procaryotes or eucaryotes) are they alive?
- replication occurs in h_____
- structure
 - c_____ of nucleic acid (could be double or single
 - stranded, DNA or RNA) surrounded by protein coat (capsid)
- main shapes
 - h_____
 - p_____
 - c_____







FIGURE 2.19 Basic morphology of protozoa. (A) scanning electron micrograph of a flagellar protozoa, Giardia; (B) scanning electron micrograph of testate cilia, *Heliosmia*; (C) electron micrograph of ciliate Didinium, Part A reprinted with permission from Cox (1993). Parts B and C reprinted with permission from Sleigh (1989).



FIGURE 2.15 Structural types of hyphae that entwine together, resulting in a mycelium.



replication

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- A______ virus adsorbs to specific receptors, receptors can be polysaccharides, proteins, or lipoproteins
 - E______ various particle or nucleic acid material enters cell
- E______ capsid is stripped away, releasing genetic material
- M______ viral nucleic acids are replicated using machinery of host cell
- M______ protein coat is synthesized and combined with nucleic acid to form nucleocapsid
- R______ of mature virions host cell ruptures release active viruses

Virus Detection and Enumeration

- animal i______ newborn mice injected with inoculum and observed for signs of disease
- t_____ cultures viruses quantified by measuring effect on host cell lines forming a monolayer on glass or plastic assay bottles, effect is measure by
- p_____ assay virus is placed on surface of host cell monolayer, virus replication leads to localized area of cell destruction called plaques
- s_____ dilution endpoint virus suspension is diluted serially and the highest dilution (smallest amount of virus) that causes a cytopathic effect in 50% of samples is reported as the tissue culture infectious dose (TCID₅₀)
- most p_____ number serial dilutions placed in tubes or microwells with host cells, positive tubes are recorded and MPN value computed from standardized MPN table.
- Rapid Detection Methods
- immunoelectron m______ viruses are incubated with specific antibodies and examined by electron microscopy for the presence of virus particles
 - immunof ______ flourescent marker is included on antibody
- e_____-linked immunosorbent assay (ELISA) antibody is fixed on solid support and virus (antigen) is added, an enzyme labeled antibody is added to the fixed antigen, presence of virus is detected by a colored product after substrate (specific for enzyme) is added
 - r_____immunoassay antibody is labelled with a radioistope



• n