Discover the World of Microbes, Bacteria, Archaea, and Viruses Gerhard Gottschalk Study Guide: Questions and Answers

#### Section 1 Batch and continuous culture

- What is the difference between generation time g and doubling time t<sub>d</sub>? g is the time required for doubling the number of cells, whereas t<sub>d</sub> is the time required for doubling the cell mass.
- Describe the characteristic feature of the logarithmic growth phase. The logarithmic growth phase is characterized by a linear increase of the logarithm of the cell number with time. It is the phase of fastest growth of a microbial species under the given conditions.
- 3. What are the features of chemostats?

A chemostat allows continuous growth of a microbial population in a vessel in which the culture volume is kept constant by a continuous influx of medium and a continuous efflux of culture broth. It is a characteristic of a chemostat that one component of the growth medium (e.g., glucose as the carbon and energy source) is growth-limiting and that the rate of inflowing medium is in a range allowing the microorganisms to completely consume the growth-limiting component (glucose in our example). Then the cell number in the culture vessel will remain constant as long as the chemostat is running properly.

4. What is the difference between oxygenic and anoxygenic photosynthesis? Anoxygenic photosynthesis proceeds without the evolution of oxygen. The reducing power required for the conversion of carbon dioxide into cell mass is provided by the oxidation of reduced sulfur compounds or of molecular hydrogen (most purple and green sulfur bacteria), or by the oxidation of certain organic compounds, such as ethanol or organic acids (other phototrophic bacteria). Oxygenic photosynthesis is much more complex. It is performed by cyanobacteria, algae green plants. The reducing power for the conversion of carbon dioxide into cell mass is generated by the oxidation of water to molecular oxygen. 5. How is chemotrophy defined?

It is the kind of living in which metabolic energy is derived from chemical reactions.

 You see rods while viewing a sample through the microscope. Name two bacterial species that could be present in the sample. The rods could be cells of *Escherichia coli* or *Bacillus subtilis*.

# Section 2 Molecules that make up microbes

- Define an informational macromolecule An informational macromolecule consists of a defined sequence of building blocks (e.g., the base sequence of DNA); the sequence contains biological information.
- 2. Why does A pair with T and G with C?

Base pairing is achieved by hydrogen bonds; their formation is most favorable between A and T (two hydrogen bonds) and G and C (three hydrogen bonds).

- 3. What is a hydrogen bond?A hydrogen of an amino group is attracted by electronegative double-bonded oxygen or triple-bonded nitrogen
- 4. What are the functions of the three types of RNA?

Ribosomal RNAs are constituents of the ribosomes (5S, 16S, and 23S rRNA).
Transfer RNAs (there are more than 20 different tRNAs in a microbial cell) are specific for the 20 different amino acids and guide these amino acids to the process of translation at the ribosomes. Messenger RNA transfers the genetic information from the DNA to the protein-synthesizing machinery.
Small non-coding RNAs are also present, they have regulatory functions (see Section 8,6)

5. Why does the genetic code comprise 64 triplets?
 The triplets are formed by the four different bases, so there are 4<sup>3</sup> = 64 different triplets possible.

- What is the function of an aminoacyl-tRNA synthetase?
   This enzyme specifically links a particular amino acid to its cognate transfer RNA (e.g., alanine to alanyl-tRNA).
- 7. What is the function of the cytoplasmic membrane and what are the constituents of a phospholipid molecule? The cytoplasmic membrane is the osmotic barrier between the inside and the outside of cells. It is electrically charged, negative inside and positive outside. The backbone is composed of glycerol molecules. They are esterified with two longchain fatty acids and with a phosphate residue that carries a positively charged molecule such as trimethylethanol ammonium. Archaea contain etherlipids instead of phospholipids (see Section 4).
- 8. What is the difference between Gram-positive and Gram-negative cell walls? Gram-positive cell walls consist of a thick peptidoglycan (murein sacculus). Gramnegative cell walls contain a thin murein sacculus that is surrounded by the socalled outer membrane.
- Describe ATP-requiring reactions.
   Kinase reactions such as hexokinase or synthetase reactions such as glutamine synthetase.

## Section 3 Evolution, from the RNA-world to the tree of life

- What are the features of the RNA world? RNA is the only informational macromolecule exhibiting both replicase activity and ligase activity. An RNA ligase would catalyze the joining of RNA fragments.
- Give several reasons why *Thermoproteus tenax* or *Clostridium ljungdahlii* could be microorganisms of the LUCA type.
   Both organisms are anaerobes. *Thermoproteus tenax* is a hyperthermophilic archaeon, able to gain metabolic energy from the formation of hydrogen sulfide from molecular hydrogen and elemental sulfur. It is able to convert carbon dioxide

into cell mass. Such an organismal type could have evolved in an anaerobic hot environment. *Clostridium ljungdahlii* is mesophilic. It grows by the formation of acetate from molecular hydrogen and carbon dioxide, but also utilizes carbon monoxide. Thus, this organismal type or its thermophilic relatives could have evolved during LUCA times. Both species utilize organic substrates.

3. What are the advantages of using 16S rRNA for the construction of phylogenetic trees?

16S rRNA is present in all prokaryotes. The molecule of about 1500 nucleotides has highly conserved sequences and regions of variability. Mutations in these regions serve as an evolutionary calendar.

4. Name the microbial milestones associated with the following microorganisms: Lactobacillus bulgaricus: biotechnology, production of yogurt. Clostridium acetobutylicum: biotechnology, production of acetone and butanol. Chromatium okenii and Chlorobium limicola: purple and green sulfur bacteria performing anoxygenic photosynthesis.

Desulfovibrio vulgaris and Archaeoglobus fulgidus: a bacterial and an archaeal representative of organisms performing dissimilatory sulfate reduction. *Streptomyces griseus*: discovery of Streptomycin.

*Mycobacterium tuberculosis*: the bacterium causing tuberculosis.

*Prochlorococcus marinus*: most likely the most numerous living organism on earth. *Anabaena azolla*: a cyanobacterium living as  $N_2$ -fixing symbiont in sea weed.

- How long did it take for oxygen to accumulate in the atmosphere after the appearance of oxygenic photosynthesis? It took about 1.5 billion years
- 6. Summarize the reactions by which reactive oxygen species (ROS) are detoxified in organisms.

The two important enzyme systems are superoxide dismutase and catalase, some of the most reactive oxygen species react nonenzymatically with organic molecules in the cell. 7. What is the principle of the Ames test?

The effect of certain compounds on mutation rates is determined. The increase of the mutation rate is a certain measure of a possible carcinogenic effect of the compound tested.

# Section 4 Archaea

- Summarize the differences in composition of archaeal and bacterial cells. Membranes and cell walls are different (see p. 281, paragraph 1). Also, components of the transcription and translation apparatus are different (see p. 288).
- 2. Name representatives of the crenarchaeota and the euryarchaeota. The hyperthermophiles belong to the crenarchaeota, including species such as *Thermoproteus tenax*, *Pyrodictium occultum*, and *Pyrolobus fumarii*. The most predominant group of microorganisms of the euryarchaeota are the methanogens, with *Methanosarcina mazei*, *Methanothermobacter thermoautotrophicus*, *Methanosphaera stadtmanae*, and *Methanocaldococcus jannaschii* as representative species. However, extremophiles such as *Picrophilus torridus* also belong to this phylum.
- 3. Which archaea hold the record with respect to maximal growth temperature? *Pyrolobus fumarii* and *Pyrodictium occultum*.
- 4. Where would you take samples to isolate *Haloarcula marismortii*? In the Dead Sea or in other salt lakes.
- 5. Which methanogens reside in our intestinal tract? *Methanobrevibacter smithii* and *Methanosphaera stadtmanae*.
- Describe the archaea involved in the sulfur cycle *Archaeoglobus fulgidus* as a sulfate reducer. Sulfolobus sulfataricus and related species as sulfur oxidizers. Thermoproteus tenax as a sulfur reducer.
- 7. Formulate the equations mentioned in the text for conversion of methanol to

methane and carbon dioxide

Consider that the oxidation of one methanol to one carbon dioxide gives the reducing equivalents for the reduction of three methanols to methane.

8. What are the differences in energy metabolism between the methanosarcinas and methanogens growing exclusively on hydrogen and carbon dioxide? Methanosarcina species contain cytochromes and methanophenacin, they couple heterodisulfide reductase with proton translocation from the inside to the outside (see Figure S13a). The only energy-conserving reaction during methanogenesis from carbon dioxide plus hydrogen by other methanogens is the methyl transferase reaction in which the transfer of the methyl group from one coenzyme to another is coupled to the translocation of sodium ions (see reaction 4 in Figure S13b).

### Section 5 Bacterial diversity

 What is the approximate upper temperature limit for growth of bacteria and archaea?

95 °C for bacteria (Aquifex aeolicus) and 113 °C for archaea (Pyrolobus fumarii).

2. Why are cyanobacteria pioneer organisms?

They are pioneer organisms because they perform an oxygenic photosynthesis and convert carbon dioxide into biomass. In addition, they do not require complex nitrogen compounds because they are able to fix atmospheric nitrogen and to convert it to ammonia and further to organic nitrogen-containing cell constituents.

- 3. What are fundamental differences between Firmicutes and proteobacteria? The major difference is that Firmicutes comprise Gram-positive bacteria and proteobacteria Gram-negative microorganisms. Endospore formers such as clostridia and bacilli belong to the Firmicutes. The proteobacteria are subdivided into  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ , and  $\varepsilon$  proteobacteria. The enterobacteria belong to the  $\gamma$  branch.
- What are anaerobic primary producers?
   Methanogenic archaea growing on hydrogen plus carbon dioxide and homoacetogenic bacteria producing acetate from carbon dioxide and hydrogen.

- 5. Why aren't the major cycles completely closed and where are leaks? Leaks are the oceans and deeper layers of soil. Organic material present there (detritus) will not be recycled in the foreseeable future.
- 6. Where does dipicolinic acid occur? The calcium salt of dipicolinic acid is an essential constituent of endospores. It is important for the heat stability of spores and also for its refractability when viewed under the microscope.
- How are thymine dimers formed?
   The formation of thymine dimers from two adjacent thymine bases in DNA is induced by UV irradiation.
- What is a mismatch?
   Mismatches during DNA replication occur between G and T as well as between A and C. They are taken care of by DNA repair systems.

### Section 6 Membranes and energy

- What are the characteristic features of ABC transporters? ABC stands for ATP-binding cassette. These transporters contain two of these cassettes. ATP is bound and is hydrolyzed to drive uptake or export of compounds.
- How is PEP involved in transport?
   PEP is the phosphorylating agent in phosphotransferase systems.
- 3. What is the basis of substrate-level phosphorylation? Certain compounds generated during glycolysis contain phosphoryl groups with a high potential for group transfer. These phosphoryl groups can be transferred to ADP to give ATP. "Energy-rich" compounds of this type are 1,3-bisphosphoglycerate, PEP, or acetyl phosphate.
- 4. What is the driving force for proton translocation in the respiratory chain?

The proton motive force is generated by oxidation of NADH (in some cases also of molecular hydrogen or of succinate) via the respiratory chain. The final electron acceptors are molecular oxygen or nitrate and its partially reduced products (nitrite, NO, and  $N_2O$ ). The driving force is the potential span between the electron donor (e.g., NADH) and the electron acceptor (e.g., oxygen). Mechanistic coupling occurs in the respiratory chain.

5. What do aerobic respiration and denitrification have in common and what are the differences?

They have in common that the stepwise decrease of the redox potential of the intermediates gives rise to the generation of the proton motive force. Differences are that denitrification proceeds under anaerobic conditions and that nitrate reduction involves intermediary compounds such as nitrite, NO, and  $N_2O$ .

6. Speculate why different light-driven electron transport systems evolved in purple bacteria and in green bacteria.

The photosynthetic apparatus of the green bacteria is designed to work also at low light intensities and to allow a light-driven reduction of NAD<sup>+</sup> to NADH. In purple bacteria this reduction is a dark reaction driven, for instance, by ATP hydrolysis or by dehydrogenation reactions.

- With which reactions do methanogens pump sodium ions?
   It is a methyl transferase by which the methyl group of methyltetrahydromethanopterin is transferred to coenzyme M.
- 8. What are MCPs good for?

MCPs allow communication between the outside and the inside of the cell. They specifically interact with certain substrates, and changes of the concentration of these substrates result in a signal affecting the chemotactic response of the cell.

9. What is the rotation mode of the flagellae of enterobacteria when they swim smoothly?

The rotation mode is counterclockwise.

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- 10.What is the effect of methylation of glutamate residues of MCPs?The signal transduction caused by the MCPs towards attractants is quenched.Methylated MCPs are active against repellents.
- 11. How is the signal transferred from the sensor kinase to the response regulator? By transfer of the phosphoryl group from a phosphorylated histidine residue of the sensor kinase to an aspartate residue of the response regulator.
- 12. Develop your own hypothesis on the structure of the sensor kinase.One could think of a bow and an arrow the arrow is released by contact of the bow with a signal compound.
- 13.Why is quorum sensing called quorum sensing?The concentration of a certain signal compound has to exceed a threshold value above which it exerts certain regulatory effects.

# Section 7 Carbon metabolism

- What is the difference between the EMP pathway and the ED pathway in the relation to glucose breakdown?
   The reactions leading to the C3 cleavage products are very different. EMP pathway: glucose is converted into fructose-1,6-bisphosphate, which is cleaved by an aldolase. ED pathway: glucose is phosphorylated, and following oxidation of the aldehyde group to a carboxyl group and further reactions, 2-keto-3 deoxy-6phosphogluconate is formed, which is then cleaved by another type of aldolase. The conversion of glucose to two pyruvate molecules via the EMP pathway is coupled to the synthesis of two ATP by substrate-level phosphorylation, whereas the breakdown via the ED pathway yields only one ATP.
- 2. Which two enzymes convert the TCA cycle into the glyoxylic acid cycle? Isocitrate lyase and malate synthase.
- 3. Why is the pathway of methane oxidation of special interest in connection with horizontal gene transfer?

The coenzyme involved in the oxidation of formaldehyde to formate is tetrahydromethanopterin, which is a characteristic C1 carrier in methane-producing archaea. It can be speculated that the genetic information was acquired by the methane oxidizers by horizontal gene transfer.

- What are the prerequisites for effective oil degradation?
   Water, oxygen, nutrients, and microorganisms able to emulsify oil and to degrade hydrocarbons.
- What do vinegar and vitamin C have in common?
   Vinegar is the product of the activity of incomplete oxidizers. One step in vitamin C synthesis the oxidation of D-sorbitol to L-sorbose is performed with an incomplete oxidizer (*Gluconobacter oxidans*)
- Why would it be difficult or even impossible for an anaerobe to operate the Calvin– Benson–Bassham cycle? Anaerobes cannot afford the ATP required for the conversion of carbon dioxide into organic compounds via this cycle.
- Why are the findings described in the statement by Andrew Benson considered to be a milestone in life sciences? He described the discovery of ribulose-1,5-bisphosphate carboxylase – the key enzyme of the Calvin–Benson–Bassham cycle and also the most common enzyme on earth.
- What determines the direction in which the Wood–Ljungdahl–pathway will proceed toward acetyl-CoA synthesis or carbon dioxide formation? The availability of reducing equivalents in the form of molecular hydrogen or reduced coenzymes.
- 9. What connects the oxidative step of the alcohol and lactic fermentation with the reductive steps?

The formation and consumption of NADH.

10. Calculate the ATP yield of the fermentation of glucose to butyrate, carbon dioxide, and hydrogen.

Two molecules of ATP are produced by substrate-level phosphorylation during glycolysis; a third ATP molecule is formed in the conversion of butyryl phosphate to butyrate.

- 11. What induces the so-called shift in acetone butanol fermentation? The drop of pH below 5 resulting in the induction of acetoacetate decarboxylase.
- 12. Speculate on the industrial potential of homoacetogens.

Homoacetogens are able to utilize syngas – a mixture of carbon monoxide and molecular hydrogen. This is important because syngas is the cheapest source of carbon and hydrogen. Homoacetogens may be genetically modified to produce ethanol instead of acetate, and also butanol and maybe other products of industrial interest.

# Section 8 Regulation of microbial metabolism

- What could it mean if one organism has two ribosomal RNA gene clusters on its genome and another organism has 10? It could mean that the organism with 10 clusters grows faster. It may make more ribosomes per cell.
- What does δ32 do in *Escherichia coli*?
   δ32 is activated in *Escherichia coli* at elevated temperatures, at 41°C. It then binds to the RNA polymerase core enzyme and promotes synthesis of messenger RNA for the production of heat shock proteins.
- **3.** Describe a system regulated by negative control.

The classical system is the *lac* operon. Its expression is blocked by a repressor that is removed by the inducer allolactose. The transcription can then proceed. It is a rather weak transcription; look up the activation mechanism under the conditions of glucose shortage.

- 4. What does the Fnr protein sense and which reactive groups are present in Fnr? Fnr senses molecular oxygen. The active form of this protein is a dimer. In the presence of O<sub>2</sub> the iron–sulfur clusters of Fnr are oxidized; the dimers dissociate into monomers that no longer bind to DNA.
- 5. Why is the regulation of amino acid synthesis so important and how is overproduction prevented at the transcriptional and the translational levels? The 20 amino acids required for protein synthesis have to be provided in the amounts needed at the ribosomes. Overproduction of individual amino acids is prevented by repression. The amino acid overproduced blocks the synthesis of the first enzyme of its biosynthesis pathway. A second regulatory mechanism is called attenuation. Ribosomal activity at the messenger RNA is attenuated if there is plenty of the amino acid around. It occurs in *Escherichia coli* and other Gramnegative bacteria. Also the activity of enzymes involved in amino acid biosynthesis is under control. The mechanism is called feedback inhibition.
- 6. What does regulation by antisense mean? It means that small RNAs bind to complementary sequences at the messenger RNA and prevent translation.
- 7. What are allosteric effectors?Allosteric effectors are structurally not related to substrates or products of an enzyme reaction. They bind to the enzyme protein and modulate its activity.
- Give an example of regulation by covalent modification.
   A good example is isocitrate dehydrogenase of *Escherichia coli*, the activity of which is regulated by phosphorylation and dephosphorylation.

### Section 9 Genomes, genes, and gene transfer

 Are the terms "genome" and "replicon" synonyms, and what is metagenomics? The genome is the total of the genetic information of an organism. It may consist of several replicons (e.g., of a chromosome and of two plasmids). Metagenomics refers to the sequence analysis of all DNA (chromosomes, plasmids, fragments) present in a particular habitat (e.g., in a certain soil, in a water sample or on the skin).

- How is it possible to identify genes in a particular organism that have been acquired by horizontal gene transfer?
   DNA regions acquired by horizontal gene transfer often differ in their GC content as compared to that of the main parts of the chromosome. These regions are called genomic islands.
- 3. What is the GC content of DNA and how does it vary among different microbial species? Double-stranded DNA consists of GCs and ATs. The GC content is expressed in

mol percent. It may vary between approximately 23 and 72%.

- 4. Summarize the three mechanisms of gene transfer.The three mechanism are transformation, conjugation, and transduction.
- 5. How is the DNA taken up by a particular organism incorporated into the genome? It is incorporated by site-specific recombination or by generalized recombination.

# Section 10.1 Antibiotics

- Describe the key observation made by Alexander Fleming.
   Fleming observed that on one of his Petri dishes a mold had grown and that bacterial growth around this mold was inhibited.
- What is the definition of an antibiotic and who coined it?
   Selman Waksman coined this terminology for compounds produced by microorganisms and capable of inhibiting growth of microorganisms or killing them.
- 3. Why do β-lactam antibiotics preferentially act on Gram-positive bacteria? They interfere with the synthesis of peptidoglycan, which is the major constituent of Gram-positive cell walls.

**4.** Describe the experimental procedure you would use to isolate an antibioticproducing microorganism.

The principle is depicted in Figure 45. Agar in a Petri dish contains enough bacterial cells so that during incubation a bacterial lawn develops on the Agar surface. Inhibitory effects of compounds can be readily observed.

- How would you test the specificity and activity of the antibiotic in question? According to Figure 45.
- Describe several targets for antibiotics.
   Targets are peptidoglycan in cell walls, ribosomes, and DNA.
- Describe the features of resistance plasmids.
   See Figure 47. A resistance plasmid contains the genetic information for decomposing, inactivating, or exporting antibiotics, and, furthermore, information for plasmid replication and transfer by conjugation.

### Section 10.2 Biotechnology

- Describe the features of a microorganism capable of producing insulin. It has to be taken into account that insulin consists of two amino acid chains connected by disulfide bridges. The corresponding genetic information has to be introduced into a production strain on a plasmid for instance, which also encodes resistance against a certain antibiotic. After overexpression of the insulin chains, the polypeptides have to be isolated and combined to give insulin.
- 2. Which parameters have an important effect on glutamate production by *Corynebacterium glutamicum*?

The critical factor is the biotin concentration. A high production rate of glutamate is only achieved at a certain concentration of biotin.

Why is biotechnology of importance for the laundry?
 Proteases are indispensable constituents of detergents.

- **4.** Which biodegradable polymers may be able to replace plastic materials? Polyhydroxyalkanoates, polylactic acid, and related polymers.
- 5. Which contribution would you expect of bioethanol and butanol towards fulfilling our energy demands?

No compromises should be made regarding our ability to feed the world and to preserve environmental quality, so it depends on the development of processes to employ, for instance, hemicelluloses or carbon monoxide and molecular hydrogen as substrates for biofuel production. Read the statement by Douglas Clark on p. 113.

### Section 10.3 Pathogenic microorganisms

- Which microorganisms like to settle in the respiratory tract? Microorganisms that have the Greek word "pneumon" for lungs in their name, such as Streptococcus pneumoniae, Chlamydia pneumoniae, Mycoplasma pneunomiae, and Legionella pneumophila. Furthermore, Mycobacterium tuberculosis and Bacillus anthracis.
- What is the potential hazard of soil?
   Spores of pathogenic bacteria could be present, e.g. of bacteria such as *Clostridium tetani*.
- What effects do the toxins of *Vibrio cholerae*, *Salmonella*, or enterotoxgenic *Escherichia coli* strains have in the gastrointestinal tract? They cause a massive efflux of water and minerals from the cells in the intestine. This loss completely upsets the body's balance of water and minerals.
- Why is it so difficult to combat *Mycobacterium tuberculosis*? *Mycobacterium tuberculosis* grows inside the cells; it also may persist for years in an apparently dormant state.
- 5. What is the infection strategy of *Pseudomonas syringae*? *Pseudomonas syringae* forms a protein that is inserted into the outer membrane of

the cells and serves as a nucleating center for ice crystals. At freezing temperatures, these ice crystals cause frost damage of the plants and allow *P. syringae* to invade.

6. Which compound serves as growth substrate for microorganisms on leaves? It is methanol, which is an intermediate in the synthesis and degradation of methyl esters in plants. Some of it diffuses out through the leaf surface where it is picked up by the so-called methylotrophic bacteria.

### Section 10.4 Viruses

- Interpret the statement of David Baltimore. As part of the infection cycle, retroviral DNA is incorporated into the host DNA and consequently also into human DNA. Eight percent of the human genome is genetic material of retroviral origin.
- Why are viruses not considered to be living organisms? They lack an independent metabolism and can only proliferate by occupying the metabolic apparatus of host cells.
- Interpret the importance of the chemical synthesis of the polio virus.
   For the first time, virus particles where generated from a chemically synthesized DNA. This so-called complementary DNA was transcribed into the polio virus genome, which is an RNA molecule, and incubation of this RNA in a cell extract resulted in the generation of polio virus particles.
- 4. What is the basis for referring to influenza viruses as H1N1 or a similar term? H stands for hemagglutinin and N for neuraminidase, which are the capsid proteins of influenza viruses. They are subject to mutations and give rise to various virus types (e.g., H1N1 or H5N1).
- Why is replication accuracy a problem in RNA viruses?
   RNA-dependent RNA polymerase does have a proofreading device; it makes about one error per 10 000 base replications.

# 6. Define a retrovirus.

The genome of a retrovirus consists of two single-stranded RNA molecules. This retroviral RNA is transcribed into DNA by the so-called reverse transcriptase. Thus, in contrast to all other viruses, proliferation starts with the conversion of RNA into DNA.

7. How are viruses employed in gene therapy?

The strategy is to insert the therapeutic gene into the genome of a virus (e.g., of an adenovirus or a retrovirus). These viruses are made incompetent of replication, but they deliver their DNA to the host, and their therapeutic gene may be expressed.

8. Give reasons why DNA may have evolved in viruses from which it subsequently entered the microbial world.

The hypothesis is that DNA evolved in viruses as the most extensively modified form of RNA, so it evolved to escape the RNA-destroying enzymes of their victims. Since DNA has several advantages as compared to RNA, it was adopted by other viruses and finally by microbial cells.