虽说考试以前声称要换题库，但是就考试实际来看还是以这个版本里的题目为基础，只是有些题改成了选择和判断而已。
还有就是如果那本课本读不完的话，尽量找一些全面的资料做一个了解性的阅读吧。精要速览很不错的，这样有很多细节性的基础题不会出错。
总之是要尽心准备。每道题尽量对照原文理解清楚了。这里我对某些题与其它知识的联系作了一些注解，仅供参考。但愿对各位的考试有所帮助。注意里面的有些章节我们考试的时候没有要求，就删去了。
GMC

欢迎与我联系，虽然我们已经离开了这个令人留恋的校园。
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目录

24. List several functions for the outer wall layer in Gram-negative bacteria?
25. What is the bacterial periplasm?
26. What types of Bacteria have a periplasm and of what significant is the periplasmic space?
27. Why sucrose is able to stabilize bacterial cells from lysis by lysozyme?
28. Describe the structure and function of a bacterial flagellum.
29. What is the energy source for the flagellum?
30. What types of cytoplasmic inclusions are formed by prokaryotes?

3. Nutrition, Growth and its control

31. Macro- and micronutrients? Defined medium and complex medium?
32. What are growth factors?
33. What environmental factors affect growth of microorganisms?
34. How to prepare a medium for cultivation microorganisms?
35. How to obtain a pure culture? How to calculate cell growth?
36. How cells divide themselves? How to do viable count?
37. What is the microbial cell growth cycle?
38. How to maintain exponential growth?
39. How to name microorganisms that grow best at a temperature?
40. How do you you name microorganisms having various O2 tolerance?
41. What enzymes are involved in detoxifying toxic active oxygen?
42. What are the physical- and chemical ways to halt microbial growth?
43. On what sites do antibiotics act on cells?
44. How to preserve food?

4. Microbial Molecular Biology

1). DNA Replication
45. Describe the DNA replication process.
46. What proteins are involved in initiation of DNA synthesis (DNA replication fork)?
47. How do leading- and lagging strands replicate?
48. What are enzymes involved in replicating the lagging strand?
49. What are the differences between replicating circular DNA and linear DNA?

2). Questions on RNA
50. What structural roles has RNA played?
51. Describe the transcription process?
52. How is transcription terminated?
53. What is a promotor and what is its function?
54. Polygenic or polycistronic mRNA?
55. What is an Operon?
56. Describe the RNA processing?

57. What is a ribozyme? For example?

3). Translation

58. Describe the translation process.
59. What are the differences between prokaryotic and eukaryotic ribosomes?
60. What are involved in initiation o protein synthesis?
61. What are polysomes and its function?
62. What is the function of molecular chaperones?
63. Start codon and stop codons?
64. Universal codons?
65. Open Reading Frame (ORF)?
66. Bioinformatics?

5. Virus

Viruses 1:

67. What are viruses? How to classify viruses?
68. How are viruses different from bacteria?
69. Describe viral structures.
70. What are two common symmetry structures?
71. What are the most common enzymes brought with by viruses themselves ? How to grow viruses?
72. How to quantify viruses?
73. What is the virus life circle?
74. How host cell protect themselves from virus attacks?
75. How do minus, plus or ss-, ds DNA viruses replicate?
76. What are the major bacteriophages? Describe Phage fC174 and its genome.
77. Describe RNA Bacteriophage MS2 and its genome.
78. What is rolling circle replication?

Virus 2:

79. What are the distinct properties of the Single-Stranded Filamentous DNA Bacteriophages M13?
80. Describe how much you know about the Double-Stranded DNA Bacteriophage: the first discovered phage and the most extensively studied ds DNA viruses T7
81. How does T7 replicate its DNA, What are the unique point of T7 transcription?
82. How the Large Double-Stranded DNA bacteriophages: the T-even Phages T4 replicate their genomes?
83. What are lysogeny? prophages? and provirus?

84. Describe lambda genome? How are lytic pathway and lysogeny pathway controlled?
85. How much do you know the transposoble virus Mu?
86. List the DNA and RNA animal viruses you know.
87. What are the consequences of virus infection in animal cells?
88. How many Positive-Strand RNA Animal Viruses you know?, Negative-Strand RNA Viruses?
89. Describe teh unique properties of influenza viruses.
90. How do retroviruses replicate themself? What are their uniquenesses? What functions do reverse transcriptase have?
91. How +, - strand RNA viruses replicate? How +, -, or ds strand DNA viruses replicate?
92. What are viroids and prions? Desrcibe whatever you know about these two unique viruese.

6. Microbial Genetics

93. Describe as much as you know about plasmids.
94. What is the difference between a plasmid and an episome?
95. What are Hfr strain? F+ or F-, or F' strain?
96. Draw the F plasmid and describe functions of various DNA regions.
97. Why is it said that conjugative plasmid contributes to evolution?
98. How many types of plasmids and their functions you have learned?
99. Schematically describe R100 plasmid and its functions.
100. How do R plasmids inactivate antibiotics?
101. What is an engineered plasmid?
102. What points do F plasmid provide to its host?
103. How to detect genetic recombination. Please cite one example.
104. How is bacterial genome mapped? What are the three types of transposable elements?
105. Explain transposon mutagenesis and its possible application.
106. Give an example to explain conversible DNA and phase variation.
107. What techniques are used to map bacterial DNA genome?

7. Industrial Microbiology

108. What major industrial products have been produced by microbial fermentation processes?
109. What steps are required for the development of an industrial microbial fermentation process?
110. How to obtain a strain for our fermentation process development?
111. How to carry out a lab scale fermentation process?
112. Why do we need to use shake flasks for lab scale process development? What can we obtain from shake flask experiment?

113. What is scale up? Why do we need a pilot experiment before we move to industrial scale production?
114. How many fermentation processes do you know? describe them!
115. What paramenters must be monitored during fermentation?
116. Schematically describe a fermentor.
117. What is the most critical problem during process scale up in microbial fermentation?
118. How to maintain a constant dissolved oxygen concentration during the fermenation process?
119. How to make high-fructose corn syrup?
120. How many ways can you use to immobilize enzymes or cells?
121. Do you know how vinegar, citric acid, yeast are made?
122. Describe how the following alcoholic beverages are made: wine, dry wine, sweet wine, fortified wine, sparkling wine, whiskey, brandy, rum, vodka?
123. How mushroom is grown?
124. What is the most critical problem during process scale up in microbial fermentation?
125. How to maintain a constant dissolved oxygen concentration during the fermenation process?
126. What are the strengths and weaknesses of a technological process.
127. How to develop a fermentation process for antibiotic production?
128. What will you do if you are assigned as a chief engineer to develop a fermentation-based project?

8. Genetic Engineering

129. What are the concepts of genetic engineering and gene cloning?
130. Describe the gene cloning procedure.
131. Schematically describe the Plasmid pBR322.
132. How to clone and express a foreign gene in pBR322?
133. How to use a modified lambda phage as cloning vector?
134. What is a cosmid? a YAC? an expression vector and secretion vector? a shuttle vector?
135. What properties an organism should have in order to be used as a host for expressing foreign genes?
136. How many prokatyotic and eukaryotic hosts for expressing foreign genes you have known?
137. What vector can carry the largest foreign genes? the smallest? (YAC, Plasmids, Lambda, Cosmid)
138. What is a phagemid?
139. What are the methods to find the right clones?
140. Describe PCR method!
141. What is the principle behind DNA fingerprint?

142. Describe cloning and expression of mammalian genes in bacteria using mRNA route and protein route.
143. How to carry out the in vitro and site-directed mutagenesis?
144. How to disrupt a gene using cassette mutagenesis?
145. How many products have been produced using genetic engineering techniques?
146. Describe how to carry out plant genetic engineering.

9. Microbial ecology

147. What is Microbial Ecology and What do microbial ecologists study?
148. What is a microevironment?
149. Describe biofilms and their functions and significance in practical applications.
150. What are the factors affecting microbial ecology?
151. List methods commonly used to study microbial ecology.
152. What is the Winogradsky column and the bacteria enrichment technique?
153. What is the Winogradsky column used for? Schematically draw it.
154. How to carry out isolation of anaerobic bacteria in pure culture?
155. How to do Identification and Quantificationof environmental microorganisms using Nucleic Acid Probes, Fluorescent Antibodies, and Viable Counts?
156. How to do the Measurements of Microbial Activity in Nature using radioisotope technique and microelectrodes?
157. What Stable Isotopes are used in Microbial Biogeochemistry? How to decide the biogenic or abiogenic of a material?
158. What are aquatic habitats and primary producers? How do they distribute in nature?
159. What is Biochemical Oxygen Demand (BOD)?
160. How are microbial activities looked like in various layers of soil?
161. What are the conditions under deep sea? What microorganisms inhabit in deep sea?
162. What makes microorganisms under deep sea so unique compared with normal microbes?\
163. Why can tube worms live under deep sea where little organic nutrients are available?
164. List microorganisms you have known that tend to live in hydrothermal vents?
165. Schematically describe carbon and oxygen cycles.
166. What microorganisms are involved in C and O cycles?
167. How is methane produced from complex organic materials via microbial activities?
168. What are primary and secondary fermenters in methane production?

169. List two syntrophic microorganisms and describe a bit.
170. How ruminants digest cellulose and starch?
171. Schematically describe nitrogen and sulfur cycles and microorganisms involved.
172. What bacteria is best understood in Bacterial iron reduction and oxidation.
173. Describe the microbial leaching process for recovering copper from CuS.
174. Why some microorganisms are resistant to mercury?
175. Microorganisms are sometime used for cleaning of environmental pollutions, such as petroleum and pesticides?
176. What is lichen and mycorrhiza?
177. How is Ti-plasmid transfer T-DNA from Agrobacterium to plant genome?
178. Why is Root nodule bacteria and symbiosis so important for legumes?

10. Eukarya

179. List the Eukaryotic Microorganism you have learned.
180. Describe the differences between various eukaryotic microorganisms.
181. What do algea have various colors?
182. How to classify algae?
183. List six major algae and describe their characteristics you have learned?
184. What are the differences snd similarities between fungal cell wall and bacterial cell wall?
185. List the types of fungi you have learned.
186. How to classify fungi?
187. What groups do Saccharomyces, Aspergillus, Mucor, Agaricus belong to?
188. Give an example of how cellular slime molds go through their various life cycles.
189. How do protozoa distinguish themself from algae, yeast, fungi, and slime molds
190. List five groups of protozoa we have learned.
191. Which protozoa causes the African Sleeping Sickness?
192. What is the unique properties of Paramecium?
193. Give an example of one protozoa that causes health problem.

11. Archaea

194. List the differences between Bacterial and Archaeal Cell Membrane and Cell Walls.
195. What archaea have you learned so far?

196. Where to isolate halophilic Archaea?
197. What are the physiological features of halophiles?
198. What are the three major classes of mathane-producing archaea?
199. List several species of methane-producing archaea you have learned.
200. List several unique methanogenetic coenzymes we have learned.
201. Describe the general properties of hyperthermophilic archaea you have learned.
202. Describe at least four hyperthermophilic archaea you have known.
203. There are two bacteria that exhibits hyperthermophilic tendencies. Describe them a bit.
204. What is Thermoplasma?
205. What seems to be the maximal temperature for life to exist?
206. Why it is said that archaea may be the earliest life on earth?

12. Bacteria I

207. Draw the phylogenetic tree of the major lineages of Bacteria based on 16S ribosomal RNA Sequence comparisons.
208. How many groups of purple bacteria you have learned?
209. Describe the differences between purple bacteria, green bacteria an cyanobacteria?
210. Why do purple bacteria, green bacteria and cyanobacteria have various colours?
211. Describe the difference between nonsulfur purple bacteria and sulfur purple bacteria.
212. List four purple sulfur and nonsulfur bacteria you know, respectively.
213. Describe the difference between green nonsulfur bacteria and green sulfur bacteria.
214. List three green sulfur and green nonsulfur bacteria you know, respectively.
215. What are the uniqueness in heliobacteria?
216. How are cyanobacteria grouped?
217. What is heterocyst in cyanobacteria?
218. What is prochlorophyte?
219. List three nitrosifying and nitrifying bacteria, respectively.
220. Give two species of sulfur and iron oxidizing bacteria and tell what they can do.
221. Describe the unique properties hydrogen-oxidizing bacteria have.
222. How to classify methanotrophs and methylotrophs?

Bacteria II

223. List three sulfate- and sulfur-reducing bacteria you have learned, respectively.

224. Describe two homoacetogenic bacteria you have learned and what are their physiological properties?
225. What are prosthecate bacteria? Are flagellates prosthecae? Why?
226. List two spirilla you have learned, give their Chinese names too.
227. Bdellovibrio is a group of interesting bacteria, what are their unique properties?
228. Give two names of the important spirochetes, describe the importance to study them.
229. Why is gliding myxobacteria interesting?
230. What is sheathed bacteria?
231. Tell as much as you know about Pseudomonads.
232. List at least three major genera of free-living, aerobic and nitrogen-fixing bacteria.
233. How can acetic acid bacteria be used for practical purposes?
234. Why is Zymmomonas interesting?
235. What are the major genera of vibrio and related genera? Why do some fishes emit light?

Bacteria III

236. List as many as possible the enteric bacteria you have known. What diseases they may cause?
237. What Neisseria species causes sexual disease?
238. Compare the differences and similarity among Rickettsia, Chlamydia and Viruses
239. What are the major groups of Gram-positive bacteria?
240. List two gram-positive Cocci you have learned.
241. What is the uniqueness of Deinococcus radiodurans?
242. List at least three lactic acid bacteria you have learned, both in English and Chinese.
243. What group of bacteria are gram-positive, endospore-forming bacteria?
244. Which bacteria can be used as bioinsecticides?
245. Differences between protoplasts and Mycoplasma?
246. What are the major groups of the High GC Gram-Positive Bacteria: “Actinomycetes”?
247. List two major groups of corynebacterium.
248. Where was propionibacterium fast discovered?
249. What is the bacteria that causes tuberculosis?
250. What is streptomycete so interesting?

13. Microbial Evolution

251. Why is Ribosomal RNAs used as Evolutionary Chronometers

252. Why is 16S rRNA employed to study phylogenetics rather than the smaller 5S rRNR and the large 23S rRNA in Prokaryotics?
253. How to sequence 16S rRNA from inside the cells?
254. How to identify a prokaryotic or eukaryotic organism based on 16S or 18S rRNA?
255. How to build Phylogenetic Trees from DNA Sequences?
256. Why it is said the archaea closer to eukarya than bacteria is?
257. What is signature sequence and how can it be used?

1. Introduction to Microbiology

1. What is Microbiology?

The study of microorganisms, a large and diverse group of microscopic organisms that exist as single cells or cell clusters, including viruses which are microscopic but cellular.

2. What are Microorganisms?

Microorganisms are a large and diverse group of microscopic organisms that exist as single cells or cell clusters (including prokaryotes and eukaryotes), also including non-cellular viruses.

重点在于陈述形态特征（单细胞或细胞群）以及组成种类（原核、真核、病毒）

3. What are Prokaryotes?

Prokaryotes are the cells lacking a nucleus and other organelles, Including Bacteria, Archaea, Actinomycetes, Mycoplasma, Rickettsia, Chlamydia and cyanobacteria.

原核微生物的种类（共七类）

- 细菌、古细菌；
- 支原体、衣原体、立克次氏体；
- 放线菌、固氮菌；

4. What are Eukaryotic Microorganisms?

Eukaryotic microorganisms are the cells possessing a membrane -enclosed nucleus and usually other organelles, including Algae, fungi, slime-mold and protozoa.

真核微生物的种类（共四类）

- 真菌
- 藻类
- 黏菌

- 原生动物

注意，Slime mold（黏菌）的概念
Nonphototrophic eukaryotic microorganisms lacking cell walls,. Divided into two groups
- Cellular slime mold: Cells aggregate to form fruiting structures
- Acellular slime mold: Cells aggregate to form simplymasses of protoplasm

黏菌的显著特点是无细胞壁的真核微生物。
生长在粪便或腐烂的植物上，其生命循环的特点是有一个粘液状的无定形阶段和一个多细胞的生殖阶段。

5. Structural differences between P & E cells and virus?

- E cells have membrane-enclosed structure called organelles and cytoskeleton to support and help move its internal components. P cells don't have these two structures.
  真核：膜被细胞器和细胞骨架
- E are typically much larger than P. 形态差异
- P DNA is present in bacterial chromosome and aggregates to nucleoid. They are haploid. Most P have extrachromosomal DNA called Plasmids, which are arranged in a circular fashion. E DNA is present within the nucleus to form chromosomes. They are diploid. 遗传物质
- Virus are not cells. They are not dynamic open systems and have no metablic abilities of their own. Although they contain genes, they lack ribosomes and therefore depend on the cells biosynthetic machinery for protein synthesis.
  无细胞结构，非动态开放体系，无自主新陈代谢，无核糖体，生命活性完全依赖于宿主细胞

注意：
- Nucleoid, 类核，专指原核细胞染色体中凝聚的 DNA 物质

6. What can microbiology do?
   a) As a basic biological science, it provides the most accessible research tools for probing the nature of life science.
      作用集中体现在细胞生理、生化特性的研究上。
      微生物易培养，以进行生化和遗传操作，为高等生物中细胞功能的研究提供有效模型。
   b) As an applied biological science, microbiology can deal with many large scale processes in medicine, agriculture and industry.
   从基础科学和应用科学两个角度来分析，可以适当发挥。

7. Five common characteristics of microorganisms?
   a) Small volume, large surface area
   b) Fast absorption and conversion

c) Rapid duplication and growth
d) Strong adaptability
e) widespread distribution and diversified species

1. 体积小，表面积大
2. 营养物质吸收和转化快
3. 繁殖力强，生长迅速
4. 适应力强
5. 分布广，种类多

可以将以上几点按照因果关系从上至下记

8. Populations, communities and ecosystems?
   a) The cells living in nature in association with other in assemblages are called a *population* and such populations are composed of group of related cells derived from a single parent cell.
   由同种细胞（发育自同一母体）构成种群
   构成种群的要素包括：
   1. 共同的栖息地（habitat）
   2. 物种同一性
   3. 种内关系（合作、分工等）

   b) The cells living in association with other populations are called microbial communities.
   种群间互作形成群落
   构成群落的要素包括
   1. 共同的栖息地（habitat）
   2. 物种多样性
   3. 种间关系（竞争、捕食、互利合作、共生、寄生等）

   c) An Ecosystem is composed of living organism together with physical and chemical constituents of their environment.
   生态系统的概念中重要的是非生物因素与生物因素的互作
   构成生态系统的要素：
   1. 物种、群落、种内种间关系等
   2. 非生物因素，如光、空气、水、土壤等
   3. 生物和环境因素之间的相互影响。
   4. 能量循环和物质循环的保证

注意：一个生态学通的特性很大程度上是由该系统内的微生物决定的

9. Brief history of microbiology?
   a) 1684 Antonie van Leeuwenhoek (discovery of bacteria)
   b) 1857-1864 Louis Pasteur (lactic acid fermentation, yeast alcohol

fermentation, spontaneous generation theory)

c) 1881-1884 Robert Koch (pure culture, cause of tuberculosis, Koch's postulates, cause of (霍乱) cholera）
d) 1889 Martinus Beijerinck (concept of a virus)
e) 1929 Alexander Fleming (discovery of penicilin)
f) 1953 James Watson, Francis Crick (DNA structure)
g) 1983 Luc Montagnier (discovery of HIV)

10. How are microorganisms cultivated?

We provide them with proper nutrients such as carbon source, growth factors, minerals and specific factors for different species. We also provide proper environmental conditions including proper oxygen concentration, temperature, and light.

必需营养与生长条件

Three main methods: cultivated in petri disk, shake flask, fermentor.

三种主要的培养方法：平板培养，摇瓶培养和发酵罐发酵。

11. Cellular evolution

From the comparison of ribosomal RNA gene sequences, the evolutionary framework of three phylogenetically distinct cellular lineages, the bacteria, the archaea and the eukarya is established. All three groups diverged from a common ancestral organism, the "universal ancestor". Eukaryotic microorganisms are the ancestors of the multicellular eukaryotic organisms, higher animals and plants.

细胞进化研究的两个重要发现：

- 真核细胞内的细胞器大都起源于原核细胞
- 古细菌在进化上与真核细胞更近

选择 rRNA 作为比较对象是因为：

- rRNA 基因序列的保守性高
- rRNA 基因序列内所含的信息量适当，且操作（提纯，测序等）难度较小

2. Cell Biology

12. Gram Staining procedures

procedure	步骤	phenomenon
Flood the heat-fixed smear with crystal violet for 1 min	结晶紫初染	All cells purple
Add iodine solution for 3 min	碘试剂与结晶紫在胞壁内形成复合物	All cells remain purple

Decolorize with alcohol briefly-20sec	酒精脱色，主要是从 Gram- 胞壁内洗去 Crystal-Iodine Complex	Gram-positive cells are purple Gram-negative cells are colorless
Counterstain with safranin for 1-2min	番红复染	G+ cells are purple G- cells are pink to red

13. Components of prokaryotic cells?

- Cell wall

注意，某些微生物中不含细胞壁，例如 *Mycoplasma*， *Spiroplasma* in Bacteria and *Thermoplasma* in Archaea

- cytoplasmic membrane

注意，因为原核细胞内缺乏膜被细胞器，故大量与细胞生命功能相关的生理生化反应在细胞膜上进行。此外，在一部分原核细胞中存在于光合作用和甲烷氧化相关的膜结构，但非叶绿体或线粒体。

- ribosomes

在组成上与真核核糖体有很大区别，故许多 antibiotics 的目标分子就是核糖体组成蛋白。且沉降系数不同。

Archaea 的核糖体构成与真核细胞类似。

- inclusions

PHA, glycogen, polyphosphate, elemental sulfur, magnetosomes 等

- chromosome

原核细胞中为 Nucleoid 和 Plasmids

- flagella

分为周生鞭毛（peritrichous）和丛生鞭毛（lophotrichous or polar）,由 flagellin 构成。

附注：

在 Mycoplasma 中，电镜照片和化学检测（针对 Peptidoglycan 的组分 muramic acid he diaminopimelic acid）显示胞壁的缺失。但是由于细胞膜内的 sterol 和 lipoglycan 的存在使得细胞膜的抗渗透压能力更强。Lipoglycan 与 LPS 类似，注射到动物体内后也可引起免疫反应。

14. Morphology of prokaryotes

Coccus	spherical or ovoid	球菌，单球菌或链球菌
Rod	cylindrical shaped	杆菌
Spirillum	rods forming spiral-shaped patterns	弧菌
Spirochete	tightly coiled	螺旋菌
Budding and appendaged bacteria	extensions of cells as tubes or stalks	有附属结构的细菌
Filamentous bacteria	chains of cells or long cells	细丝菌

15. Components of eukaryotic cells?
Cell wall, cytoplasmic membrane, a true nucleus, organelles (like mitochondrion, chloroplast)

16. Structure of cytoplasmic membrane?
A thin structure that complete surrounds the cell. It is Phospholipid bilayer with proteins embedded within it.

17. How are cytoplasmic membranes stabilized?
   a) hydrogen bonds and hydrophobic interaction
   b) cations (Ca^{2+},Mg^{2+}) combine with negative charged phospholipids.
   c) Strengthening agents: sterol in eukanyotic cells, hapanoids in some bacteria

18. Differences between archeal and prokaryotic membranes?
古细菌在对特殊的生存环境（高温、高压等）的适应过程中，产生了性质独特的胞质膜结构，与原核、真核细胞膜的区别在于：
- 甘油（glycerol）与支链有机酸的成键方式不同。在古细菌细胞膜内采用热稳定性和酸碱稳定性更高的醚键连接，而在原核细胞膜内则采用酯键连接。
- 磷脂分子中的重复支链结构不同。在古细菌细胞膜内重复结构为 isoprene（2-甲基丁二烯）。而在细菌细胞膜内重复结构为脂肪酸，包括饱和脂肪酸和不饱和脂肪酸。
- 古细菌细胞膜内的磷脂分子通过 glycerol diether 或 diglycerol tetraether 达到自稳态结构，无须形成双分子层即可行使功能（包括嵌套膜蛋白），故为 lipid monolayer 结构。而在细菌细胞膜疏水力驱使磷脂分子必须形成 lipid bilayer 才能达到最稳定结构。
- 古细菌膜表面嵌套的蛋白也有相应的对高热高压的适应机制，其化学修饰机制与细菌细胞膜蛋白有区别。

19. Functions of cytoplasmic membrane?
a) permeability barrier （物质通透障）
   Cytoplastic membrane prevents cell-cell leakages and functions as a gateway for transport of nutrients into and out of cell
b) protein anchor （蛋白锚定点）
   On both sides of Cytoplastic membrane there are anchor sites of many proteins involved in transport, bioenergetics, chemotaxis
c) energy conservation （能量保存层）
   The ATPase on the Cytoplastic membrane can use the proton motive force caused by conservation of protons by the membrane to generate energy.

20. Actions of carrier proteins
- Uniporter， 单转运 （A 进）

- Symporter, 共转运 （B 随 A 进）
- Antiporter，反转运 （A 进 B 出）

附注：
转运的三种方式

- Simple Transport
  物质随氢离子一同通过 Symporter 进入细胞膜，能量来自于 proton motive force, 如 lactose 的转运
- Group Translocation
  被转运物质被膜上转运蛋白进行化学修饰，该过程中释放的能量被用作物质转运。例如 glucose 的跨膜转运，就涉及到至少五种蛋白形成的级联磷酸化，导致高能磷酸键通过蛋白链传递到 glucose,释放能量，磷酸化 glucose 并完成转运。进入细胞内的 glucose 6-Pi 可以迅速进入三羧酸循环。
- The ABC system （ATP binding cassette）
  同时涉及跨膜转运蛋白（uniporter）以及 periplastic 蛋白的功能，能量来自于膜内 ATP 水解酶促进的 ATP 水解。

21. Differences in cell walls between Gram-positive and Gram-negative bacteria

G+
Gram 阳性菌的细胞壁内含有近 90% 的 peptidoglycan 及少量 Teichoic acid。其中 peptidoglycan 可能分为多层， 而 Teichoic acid 的负电性也会影响离子的通透。
G-
Gram 阴性菌在 peptidoglycan 层的外侧存在 outer membrane，其与 cytoplastic membrane 之间的空间成为 periplasma ，peptidoglycan 层位于其中。
Outer membrane 主要由下列分子构成：

- Lipopolysaccharide （LPS，其 lipid A 部分为 endotoxin）
- Protein （其中重要的通道蛋白包括 porin，孔道蛋白，允许小分子物质通过 outer membrane）
- phosphorlipid,
- lipoprotein,位于 outer membrane 内侧，起锚定作用。

22. Why is the bacterial cell wall rigid layer called peptidoglycan?
This layer is a thin sheet composed of two sugar derivatives, N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), and a small group of amino acids consisting of L-Ala, D-Ala, D-Glu and either Lys or DAP. These constituents are connected to form the glycan tetrapeptide.

注：peptidoglycan 的组成特性使其成为 Penicillin（青霉素）的目标。

23.What are the chemical reasons for the rigidity that is confered on the cell wall by the peptidoglycan structure?
The glycosidic bonds connection the sugars in the glycan chain are very strong. The full strength of the peptidoglcan structure is realized when the

glycan chains are cross-linked by amino acid.而由 peptide 实现的 crosslinking 的数目和形式会直接导致 peptidoglycan 层的厚度及硬度。

注：在 Gram+ 胞壁中，存在 peptide interbridge 使得 peptidoglycan 厚度和硬度增加。

24.List several functions for the outer wall layer in Gram- bacteria

1. 膜上蛋白作为表面抗原被免疫系统识别，产生抗体
2. LPS 对于动物有毒，会形成 endotoxin（特别是 Lipid A）
3. outer membrane 中存在孔道蛋白，对于小分子物质具有一定的透过性。但不允许酶等大分子透过，以防止 periplasma 中的酶扩散到胞外。

25.What is the bacterial periplasm?

This space between the outer surface of the cytoplasmic membrane and the inner surface of the LPS-containing outer membrane, 12-15nm, gel like.

Gram-negative bacteria contain a space between cytoplastic membrane and a lipid-rich outer membrane layer, called periplasm, which is used to keep various protein from diffusing away.

注：Gram + 中没有 periplasm，故其功能蛋白直接分泌到胞外基质中，容易扩散损失。

26. Which type of Bacteria have a periplasm and what significance is the periplasm?

G-,many important enzymes present in the periplasm，including

- hydrolytic enzymes, which function in the initial degradation of food molecules,
- binding proteins, which begin the process of transporting substrates(参与 ABC system 转运)
- chemoreceptors, which are proteins involved in the chemotaxis response.

27. Why is sucrose able to stabilize bacterial cells from lysis by lysozyme?

In a solution containing a high (proper) concentration of a solute such as glucose, the solute concentration outside the cell balances that inside, so although lysozyme still digests peptidoglycan, water can not enter the protoplast, so the lysis doesn't occur.

28. Describe the structure and function of a bacterial flagellum

Structure: filament , hook and Basal body.

Basal body is composed of L Ring (锚定外细胞膜),P Ring(锚定 periplasm 中的 peptidoglycan), S-M Ring(两个，锚定于内细胞膜), Mot protein(借助于氢离子内流的能量产生驱动力, proton motive force) and Fli protein(motor switch)。

Function: movement (mobility)

注：除鞭毛运动外，细菌中还存在 gliding mobility

29.What is the energy source for the flagellum?

Proton motive force, proton across membrance through Mot complex drives rotation of the flagellum,and 1000proton per single rotation of the flagellum.

30.What types of cytoplasmic inclusions are formed by prokaryotes?

PHA, glycogen, polyphosphate, elemental sulfur, magnetosomes

(PHB granules, glycogen granules, polyphosphate granules, sulfur granules, magnetosomes)

- Polyhydroxyalkanoates (carbon source and energy storage materials)
- Glycogen (carbon source and energy storage materials)
- Polyphosphate
- Elemental sulfur
- Magnetosomes (magnetotaxis of *Aquaspirillum magnetotacticum*)

3. Nutrition and Metabolism

31. Macronutrients and micronutrient, defined medium and complex medium

- Nutrients are starting materials for hundreds of chemical compounds present inside a living cell. Elements required in fairly large amounts are so-called macronutrient (including C, H, O, N, S, P, K, Mg, Ca, Na, Fe)(11 种)

  metals needed in very small amounts are called micronutrients. (including Cr, Co, Cu, Mn, Mo, Ni, Se, W, V, Zn, Iron Fe)(11 种)

  Micronutrients play a structural role in various enzymes.

- Chemically Defined media are prepared by adding precise amounts of highly purified inorganic or organic chemicals into distilled water. Examples are glucose, sucrose, acetate, pyruvate, malate, fatty acids, alkanes et al. Complex media employ digests of casein, beef, soybeans, yeast cells, or any of a number of other highly nutritious substances.

32. Growth factors

Growth factors are organic compounds required in very small amounts and only by some cells ,including vitamin A, purine and pyrimidine.

33.Environmental factors affect growth of microorganisms

Temperature, pH values, osmotic effects and oxygen

34.How to prepare a medium for cultivation microorganisms

Two aspects must be highlighted: one is the understanding of the nutritional

requirements the given microorganisms need, and the other is supplying essential nutrients in the proper form and proportions in a culture medium.

35.Pure culture access and cell growth calculation

- Proper nutrients and conditions ,aseptic techniques.
- By streak plate technique
- Cell growth in exponential phase can be expressed as follows:

$$N = N_0 2^n \quad \text{or} \quad n = \lg\frac{N}{N_0} \Big/ \lg 2$$

slope = lg2 / g

Population growth is measured by following changes in the number of cells or weight of cell mass:

- Total Cell Counting (direct microscopic count)——counting a sample under the microscope, samples either dried on slides or in liquid.
  无法区分菌体的活性。
- Viable Counting (plate counting or colony counting——including two methods: spread plate method（涂板法）and pour plate method（倒板法）.
  该方法只检测活菌。
- turbidimetric measurement (浊度测定)
  无法区分菌体活性。

36. How cells divide themselves and how to do viable count

- The way cell divides themselves: binary fission (most common), budding and, multiple fission (uncommon).
  均等分裂、出芽和复分裂
- a viable count is to determine the number of cells in the sample capable of forming colonies on a suitable agar medium. There are two ways to perform the task: one is so-called spread plate method, and the other pour plate method. In both methods, three steps are employed: dilution, plating and incubation.

37. Microbial cell growth cycle

Growth cycle of microbial populations can be divided into several distinct phases: lag phase, exponential phase (or log phase), stationary phase and death phase.

38.Exponential growth maintenance

This can be achieved using a chemostat by controlling the dilution and concentration of nutrients and stabilizing the conditions at the proper state for bacterial growth.

39.How to name microorganisms that grow best at a temperature?

psychrophiles	0-20
mesophiles	20-45
thermophiles	45-80
hyperthermophiles	>80
extreme hyperthermophiles	80-120

40.How do you you name microorganisms having various O_2 tolerance?

Aerobes

Obligate aerobes: oxygen presence necessary

Facultative aerobes: oxygen not necessary, but better with it

Microaerophilic aerobes: required O2 at low level than atmospheric

Anaerobes

Aerotolerant: O_2 not required, and growth no better if O2 present

Obligate (strict) anaerobes: Oxygen harmful or lethal

41 What enzymes are involved in detoxifying toxic active oxygen?

Degradation of hydrogen peroxide, H_2O_2 ：catalase, peroxidase

Degradation of superoxide, O_2^- ： catalase and superoxide dismutase

过氧化氢降解依赖于过氧化氢酶和过氧化氢物酶；

超氧化物降解依赖于超氧化物歧化酶和过氧化氢酶。

42. What are the physical- and chemical ways to halt microbial growth?

Physical:

Heat sterilization(use autoclave; 部分灭菌 pasteurization)

Radiation(microwave, UV, X-ray, r-ray, electrons)

Filtration (depth filter, membrane filter, nucleation track filter)

Chemical(germicides):

cidal agent(kill or inhibit,不可逆);

static agent(inhibit growth but not kill bacteria，可逆)

disintectants(kill microorganisms and are used on inanimate object)

antisepeics(kill or inhibit and are sufficiently nontoxic to be applied to living tissue)

体内抗菌 chemotherapeutic agent: (have selective toxicity)

1 synthetic agent: growth factor agent, quinolones

2 antibiotics

43. On what sites do antibiotics act on cells?

- Cell wall synthesis,
- folic acid metabolism,
- cytoplasmic membrane structure,
- DNA gyrase,
- DNA directed RNA polymerase,
- inhibition of protein synthesis through tRNA or ribosome (30s.50s)

目标位点	作用类型	例子

细胞壁（阻止 peptidoglycan 的合成）	Inhibition of cross-linking	Penicillin
	Inhibition of polymerization	Glycopeptide antibiotics
蛋白质合成	Inhibition of translocation of ribosome	Aminoglycoside antibiotics
	Inhibition of binding of aminoacyl tRNAs	Tetracycline (四环素)
核酸合成	Inhibition of tetrahydrofolic acid synthesis	Sulfonamides (磺胺制剂)
	Inhibition of DNA gyrase	Quinolone antibiotics

细菌中抗生素 resistance 的产生方式

Mechanism	Examples	
Antibiotic Inactivation 抗生素失活	beta-Lactamase	Penicillin resistance
	Chloramphenicol acetyl transferase	Chloramphenicol (氯霉素) resistance
	Aminoglycoside modifying enzymes	Aminoglycoside resistance
Reduction of permeability 通透性抑制	Reduced uptake (降低吸收量)	Natural resistance of Gram-bacterial due to the presence of outer membrane
	Antibiotics efflux(外排)	Tertracycline resistance
Alteration of target site 作用位点修饰	No longer sensitive to the drug(原位点脱敏)	Sulfonamide resistance
	New target sites not sensitive to the drug (新位点不敏感)	Methicillin (甲氧苯青霉素) resistance
	Overproduction of target sites(冗余)	Trimethoprim (甲氧苄氨嘧啶) resistance

44. How to preserve food?

Sterilization, lower temperature, pH acidity, low water availability, canning, chemical food preservation

灭菌、低温、强酸、脱水、装罐、化学药物处理后储存

4. Microbial Molecular Biology

45. Describe the DNA replication process.

- DNA synthesis begins at a unique location called the origin of replication.
- The double helix is unwounded by helicase and is stabilized by single

stranded binding protein.

- DNA polymerase (generally III) is then bound to each of the DNA strands. The extension of the DNA replication occurs continuously on the leading strand, but discontinuously on the lagging strand. In the leading strand(5'-－3'), replication is from 5'end to 3'end according to "complementary principle".In the lagging strand(3'－5'), replication is also from 5'end to 3'end pieces by pieces, and primer is needed. The primer is synthesized by primase.
- After synthesis by polymerase III, the primers are removed by the DNA polymerase I.
- Finally, the pieces of DNA of the lagging strand are linked by ligase.

Most errors in base pairing are corrected by proofreading functions associated with the action of DNA polymerase.

46.What proteins are involved in initiation of DNA synthesis (DNA replication fork)?

- helicases
- single-stranded DNA binding proteins

47. How do leading and lagging strands replicate?

Leading strand:

DNA synthesis occurs continuously from the 5'end to 3'end.

Lagging strand:

DNA synthesis occurs discontinuously and the pieces called Okazaki fragments are linked by ligase.

48. What are enzymes involved in replicating the lagging strand?

- DNA primase,合成 RNA primers，3'端有-OH, 可供添加新的 nucleotide
- DNA polymerase III, 去除引物，补全 DNA 链。此外还有 proofreading 功能
- DNA polymerase I，功能包括 3'-5'添加 nucleotide，5'-3' exonuclease 和 proofreading
- DNA ligase,连接新合成 DNA 链中的 nicks,包括连接 okazaki fragments.

49. What are the differences between replicating circular DNA and linear DNA?

For linear DNA replication , there is one problem: at the extreme 5'-end of each strand of linear DNA, even if the RNA primer is very short and there is one special enzyme to remove it, no DNA polymerase can replace it with DNA since all DNA polymerase require a primer. So there are many ways to solve this problem, such as sticky ends, direct repeats, using a protein primer or telomerase and so on.

注：telomerase 在 3'端添加数目不定的重复片段，其本身带有一个 RNA 模板 as a cofactor，故不需要 DNA 模板。

50. What structural roles has RNA played?

- Messenger RNA (mRNA)
- Ribosomal RNA (rRNA)
- Transfer RNA (tRNA)
- Catalytic RNA: Ribozyme

注：ribozyme 指由催化生化反应能力的 RNA 片段，如 telomerase 中的 RNA 辅因子。大多数 ribozyme 的为 self-splicing intron, 在发挥功能的时候自身也失去活性，只能发挥一次 sequence-specific endonuclease 的功能。RNaseP 为一具有特定功能结构的可重复形式功能的 ribozyme.

51. Describe the transcription process?
The initiation and termination sites are specific nucleotide sequences on the DNA. RNA polymerase moves down the DNA chain, causing temporary opening of the double helix and transcription of one of the DNA strands. When a termination site is reached, chain growth stops and the mRNA and polymerases are released.

52. How is transcription terminated?
Termination of transcription occurs at specific base sequences on the DNA. Stem-loop structure formed by intra-strand base pairing caused the release of ribosome and the termination of transcription.

53. What is a promotor and what is its function?
Promoters are specific DNA sequences at which RNA polymerase can bind and begin transcription.

54. Polygenic or polycistronic mRNA
a single mRNA molecule that often codes for more than one protein in prokaryotes.

55. What is an operon?
A series of genes all regulated by one operator is called operon
Operon is a complete unit of gene expression, often involving genes coding for several polypeptides on a polycistronic mRNA or genes coding for ribosomal RNA.

56. Describe the RNA processing.
The conversion of a precursor RNA into a mature RNA is called RNA processing.
In prokaryotes and eukaryotes, tRNAs and rRNAs are made initially as long precursor molecules, which are then cut to make the final mature RNAs.
The mRNAs of eukaryotes need three more processing steps: splicing, capping (5' end) and tailing(or polyadenylation, 3' end), and also partially methylation.

57. Describe the translation process,

Initiation: mRNA is attached to the ribosome(involving the Shine-Dalgarno sequence),forming the initiation complex. And then a special initiator aminoacyl-tRNA (fMet-tRNA) binds to the start codon:AUG.

Elongation:further more aminoacyl-tRNA is attached to the mRNA, leaving the amino acid, then a peptide bond forms.

Termination-release: when the stop codon is met, the release factors read the chain-terminating signal and serve to cleave the attached polypeptide from the terminal tRNA. Following this, the ribosome dissociates, and the subunits are the free to form new initiation complex.

Polypeptide folding: many proteins require the assistance of other protein called molecular chaperones for proper folding or for assembly into larger complexs.

59.What are the differences between prokaryotic and eukaryotic ribosomes?

	Prokaryotic	Eukaryotic
Size	70s(30s+50s)	80s(40s+60s)
Small subunit Number of proteins RNA size(number of bases)	30s ~21 16s(1500)	40s ~30 18s(2300)
large subunit Number of proteins RNA size(number of bases)	50s ~34 23s(2900) 5s(120)	60s ~50 28s(4200) 5.8s(160) 5s(120)
Other	Can use polycistronic mRNA because the ribosome can find each initiation site within a message	Cannot translate polycistronic mRNA(typical recognize an mRNA by its 5'cap and initiate only ate the 1st initiation codon)

60.What are involved in initiation of protein synthesis?

Begin with a free 30s ribosome subunit and an initiation complex forms consisting of 30 S ribosome subunit, mRNA, formylmethionine tRNA (Start codon AUG), initiation factors and Guanosine triphosphate.

61.What are polysomes and its function?

When several ribosomes are simultaneously translating a single message, the complex is called a polysomes increase the speed and efficiency of mRNA translation, and because each ribosome acts

independently of the others, each ribosome in a polysome complex can make a complete polypeptide

62. What is the function of molecular chaperones?

Molecular Chaperones assist many proteins for proper folding or for assembly into large complexes.

63.Start codon and stop codon?

Several three consecutive nucleotides coding for the initiation of protein synthesis(translation) are called start codons;

Several three consecutive nucleotides coding for the termination of protein synthesis(translation) are called stop codons;

64.Universal codons?

Universal code is the exact same code used by all living systems. (the answer is not "codons")

65.Open Reading Frame (ORF)?

a message RNA starts with a codon AUG and ends with codons UAG, UAA, or UGA.

66.Bioinformatics?

The subject combined of computer and molecular biology

5. Virus

67. What are viruses? How to classify viruses?

Virus is a noncellular genetic element that enlists a cell for its own replication. It also has a mature infectious extracellular state.

病毒是一种独立于细胞染色体而依赖于细胞进行复制的遗传因子,具成熟的胞外感染结构。

分类方法

I. According to hosts:

- bacterial viruses
- animal viruses
- plant viruses

II. According to nucleic acid structure:

- DNA viruses
- RNA viruses;
- RNA-DNA viruses

注：RNA-DNA viruses 包括 Retroviruses (ss RNA) 和 Hepadnaviruses(dsDNA)。Retrovirus 的 virion 中含有 RNA genome,但在复制过程中以 DNA 为媒介。而 Hepadnaviruses 则相反。

68. How are viruses different from bacteria?

- Viruses have smaller sizes than bacteria 体积小
- Viruses have either DNA or RNA as genetic material, single stranded, double stranded or as in Hepadnavirus partially double stranded. In

Bacteria, most of the genomes are double stranded DNA. 遗传物质基础的差异

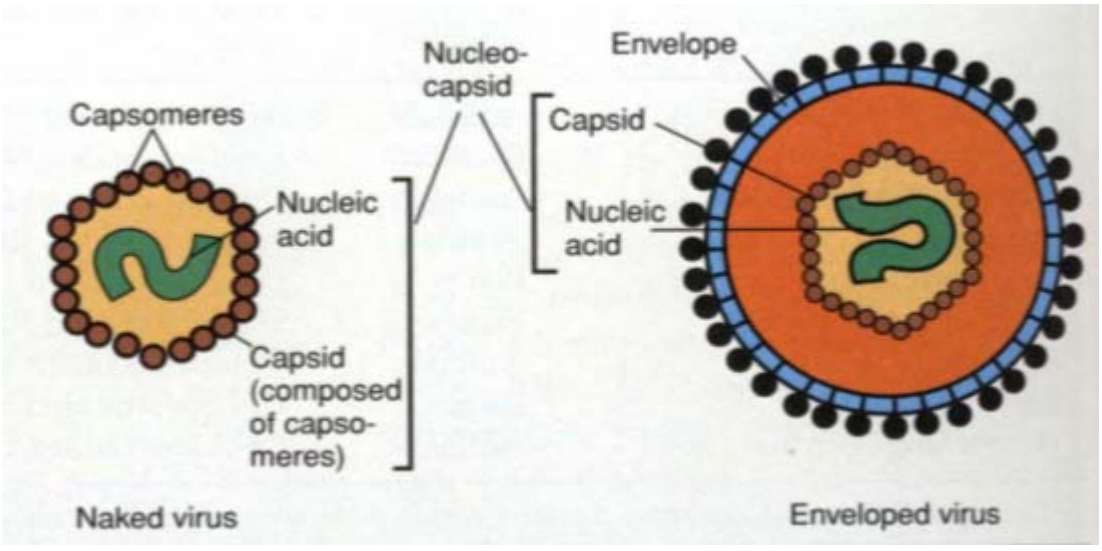
- The viruses do not have cellular structures while the bacteria have complete cellular structure, though a bit simpler than the eukaryotes.结构简单，无细胞结构
- The viruses need host cells for replication. Bacteria is a major one of their target. 宿主依赖

69. Describe vital structure.

They have nucleic acid in the capsid which is composed of capsomeres. Many have a complex membranous structure that is composed of lipid bilayers with membrane specific proteins surrounding the nucleocapsid and called an envelope. Some viruses are more complex, being composed of several separate parts with separate shape and symmetries, such as the tail, head, endplate and tail fibers in T4 bacterial virus.

注：此处还应注意两个特例

- Viroid,为 small circular ssRNA pathogenic molecules。其胞外形式为 naked RNA,不含 capsid 蛋白且 Viroid 基因组本身不编码任何蛋白质。
  注意 naked virus 与 naked RNA 的区别。
- Prion，只含有蛋白质，不含核酸的感染性病原体，可导致 BSE，CJD(in human)等。编码 prion 的蛋白位于宿主体内，在 misfold 蛋白和 molecular chaperone 的作用下，正常的蛋白被 misfold。正常情况下产生的蛋白多位于 neuron 中，故表现为精神类异常。



70. What are the two common symmetry structures?

- Helical symmetry
- Icosahedral symmetry(3 个/面，60 个/粒，同一面上三个分子可以不同也可相同)

71. What are the most common enzymes brought with by viruses themselves? How to grow viruses?

- Neuraminadase: break down glycoprotein, aiding liberation of the virus
- Lysosome: bacteriophage
- Reverse transcriptase (RNA-dependent DNA polymerase) in retroviruses

Methods of growing viruses:
Bacteriophage: bacterial cultures
Animal viruses: Tissue or cell cultures

注：细胞培养方法包括贴壁培养和悬浮培养。用于病毒研究的细胞可分为 primary cells(单细胞直接培养), diploid cells strains(培养过多个世代，5<passages<60) 和 continuous cells lines(如 Hela cells from epithelium and BHK cells from fibroblast,均采自 tumorous tissue).

72. How to quantify viruses?
Measure the viruses' effect on the host cells that they infect. By determining the number of infectious units per volume of fluid, a measure of virus quantity can be obtained.

注意两个概念：

- Efficiency of plating: 病毒感染平板细胞的效率，与环境因素，宿主和病毒特性有关。多数病毒的 efficiency of plating 低于 50%，有的甚至不到 1%。故检测病毒数目是单纯用绝对数量来衡量是不准确的。
- Plaque-forming unit (上文中的 infectious unit)：可以导致可观察细胞反应的最小数目的病毒粒数目。以此作为单位衡量病毒的数目在实际应用中更有意义。

73. What is the virus life circle?

- Attachment (adsorption) 粘
- Penetration (injection) 透
- Replication and Synthesis of protein subunits 产
- Assembly and packaging 聚
- Release 散

74. How do host cell protect themselves from virus attacks?

- 在 bacteria 中，宿主细胞主要通过限制性核酸内切酶的功能来破坏进入体内的外源 DNA（virus）。而本身的 DNA 可以通过甲基化得以保护。某些 T-even 噬菌体发育出 glycosylation of DNA 机制以保护其 DNA。
- 在高等动物体内，免疫系统 B 细胞借助于 T4 细胞可以识别病毒表面的 epitope(抗原决定基)。初级免疫反应有 IgM 和低浓度的 IgG 做出。次级免疫反应主要由 IgG 行使。

注：HIV 可以导致 Immunosuppression

75. How do minus, plus or ss, ds DNA virus replicate?

ds DNA	m RNA	protein	Assembly			Pox, herpes, irido
	dsDNA					
ss DNA	dsDNA	mRNA	Protein	Assembly		parvo
		ssDNA				
ss/ds RNA	dsDNA	mRNA	protein	Assembly		hepadna
			ss/ds DNA			
+ ssRNA	Protein		assembly			Picorna calici
	-RNA	+RNA				
- ssRNA	+RNA	Protein	assembly			Otrhomyxo rhabdo
		-RNA				
ssRNA (retro)	dsRNA	integration	mRNA	protein	Assembly	retro
				mRNA		

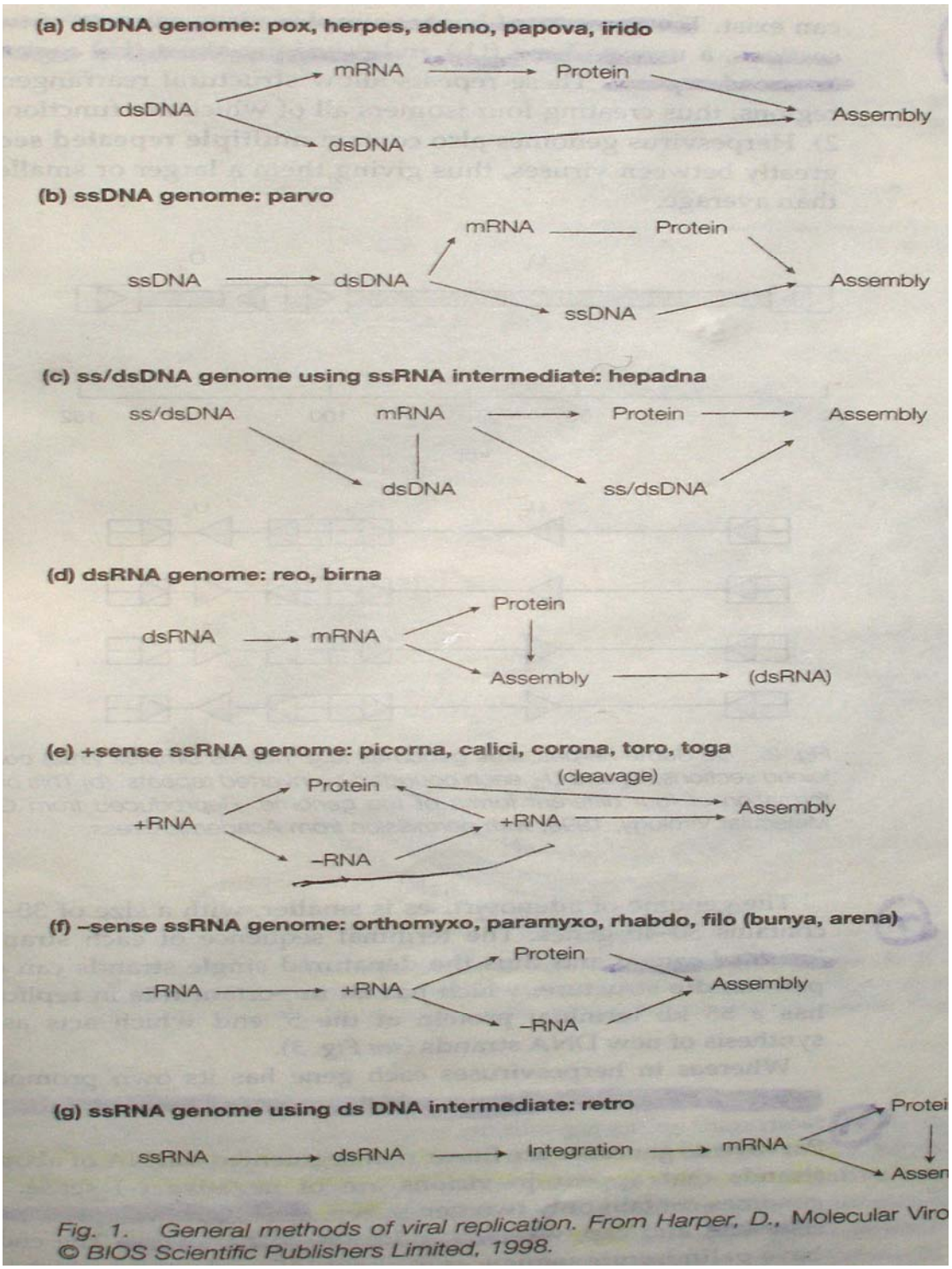


Fig. 1. General methods of viral replication. From Harper, D., Molecular Viro
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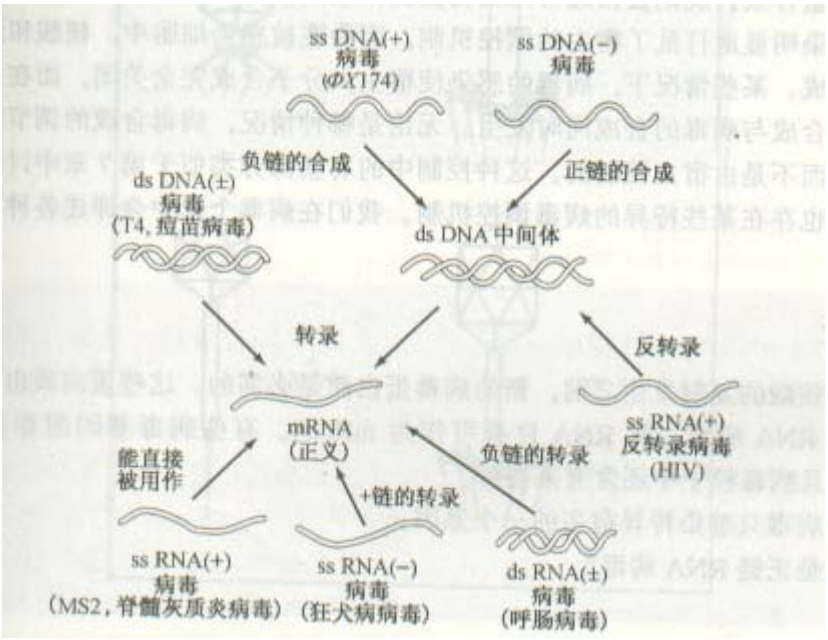


以上两幅图都是讲各种病毒如何转录至 mRNA 实现翻译的，

76. What are the major bacteriophages? Describe Phage φX174 and its genome.

- single stranded RNA viruses: MS2,
- double stranded RNA viruses: φ6
- single stranded DNA viruses: φX174, fd, Ml3
- double stranded DNA viruses: T3, T7,Mu,Lambda,T2,T4

φXI74 is a single stranded DNA virus, consisted of a circular single stranded DNA of 5386 base, Icosahedral, having overlapping gene, the first DNA virus to be completely sequenced in 1977.

77. Describe RNA Bacteriophage MS2 and its genome.

MS2 is an ss +RNA virus which is the first RNA virus to have genome sequenced in 1976. MS2 has the common characteristics of RNA Bacteriophage:

- small (26 nm) and 3596 nucleotides long
- icosahedral,
- With 180 copies of coat protein per virus particle.

78. What is rolling circle replication?

单链 DNA 复制时首先要形成 RF（replicative form），RF 为双链环状 DNA。之后 RF 中的某条链上出现缺口，缺口的 3'端被用于粘接 primer 起始转录。之后模板链不断旋转，旧的互补链逐渐离开，新的互补链与模板链互补形成 RF。当合成完成时，gene A protein 将两个互补链切开，并由 ligase 将旧的互补链两端粘结成环，完成复制。

79. What are the distinct properties of the ss filamentous DNA bacteriophages MI3（类似的还有 f1 和 fd）?

- Infect *E.coli*
- Infect only male cells through attachment to the male-specific pilus
- circular single-stranded DNA, not self-complementary
- can be used as a cloning vector and DNA sequencing vehicle in genetic engineering
- Infect but not kill the cells, release by budding process without accumulation of virions intracellular

M13 适于做测序和克隆载体的优点在于：

- 单链，可直接测序
- 不杀死宿主细胞，可以获得持续不断的病毒粒
- 内含不编码的序列可以插入大量外源基因，适于克隆

80. Describe how much you know about the Double-Stranded DNA Bacteriophage: the first discovered phage and the most extensively studied ds DNA viruses T7.

- Linear double-stranded DNA with 39,936 base pairs
- 92% DNA codes for protein
- Gene overlapping through translation in different reading frame and through internal translational reinitiation as well as internal frame shifts.
- One of the coded proteins inhibits the host restriction system, which is synthesized before the entire T7 genome enters the cells.

81. How does T7 replicate its DNA, what are the unique points of T7 transcription?

The DNA of T7 is a linear double-stranded DNA. The DNA replication in T7 begins at a single origin and proceeds bidirectionally from this origin.

- 关于引物和转录起始。T7 的转录起始点位于 DNA 中，同时在两条链上起始转录，方向不同(3'-5' 和 5'-3')。左向引物由 T7 primase 合成，右向引物由 T7 RNA polymerase 合成。在转录起始后不久形成 eye 型结构，转录到达终点后会形成 Y 型结构。
- 5'-3'方向的合成可以完整进行。但 3'-5'方向上由于引物在复制完成前即脱离，故在 5'端存在没有复制的区域。T7 的策略是在末端添加重复序列。 The opposite single 3'-strands on two separate DNA molecules, being complementary, can pair with these 5'-strands. And through the action of DNA polymerase and DNA ligase, DNA molecules are joined at the unreplicated terminal ends to form concatamers（连接物，包含两组相同的基因片段）. At last, the endonuclease cuts each concatamer at specific sites, resulting in the formation of virus-sized linear molecules with terminal repeats。
- The unique point of T7 transcription is that several genes at the left end of the DNA are transcribed immediately by the cellular RNA polymerase, one of these early proteins inhibits the host restriction system, and this protein

is synthesized before the entire T7 genome enters the cell. Two other early mRNAs code for proteins that stop the action of the host RNA polymerase. Thus the host RNA polymerase is used just to copy the first few genes and to make the mRNA that codes for the phage-specific RNA polymerase.

82. How the Large Double-Stranded DNA bacteriophages: the T-even Phages T4 replicate their genomes?
The DNA of T4 is also a linear double-stranded DNA. And the DNA replication process in T4 is similar to that in T7, but in T4, at the end of the process, the cutting enzyme that forms virus-sized fragments from concatamers dose not recognize specific locations on the long molecule but rather cuts off head-full packages of DNA irrespective of the sequence.（内切酶无法识别特定序列）

83. What is lysogeny? Prophage? Or provirus?
lysogeny: Viruses can enter a state called lysogeny, where most phage genes are not expressed, and the phage genome is replicated in synchrony with the host chromosome.
Prophage: also called provirus, is the genome of a temperate virus when it is replicating, and usually integrated into, the host chromosome.

84.Describe lamda genome? How are lytic pathway and lysogenic pathway controlled?

Lamda genome is linear double-stranded DNA, it has a Single-stranded tail of 12 nucletides long at the 5'-terminus, these single-stranded ends are complementary (cohesive) and the two ends can associate and form a double-stranded circle containing 48,502 base pairs.

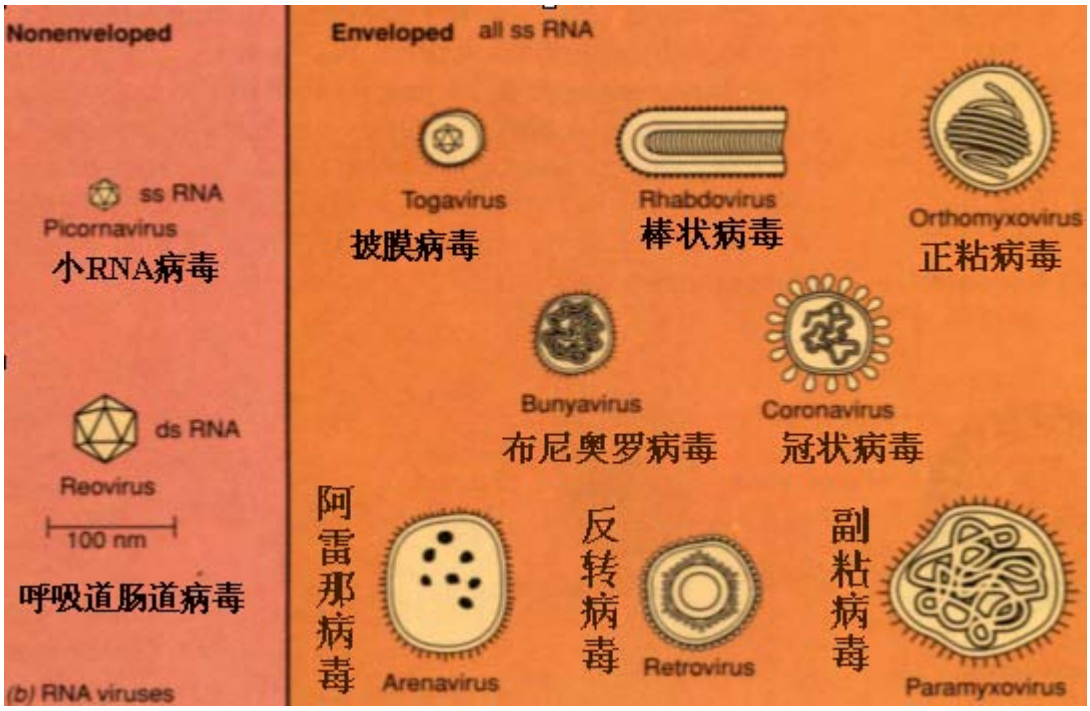
Lamda has a genetic switch that controls whether the lylic pathway or the lysogenic pathway is followed. Both regulatory proteins Cro and the lamda repressor bind to O_R(operator right) on the lamda genome to carry out regulatory functions. The Cro protein binds to the three sites of OR in the order site 3, site 2, and then side 1. The lamda repressor binds to these sites in the opposite order. PR is transcribed immediately on phage entry into the cell. Rightward transcripation from this promoter is necessary to produce Cro protein and other downstream. genes to start the lytic cycle. Leftward transcriplion from either PE or PM is necessary to synthesize the lamda repressor(product of the Cl gene) to start the lysogenic cycle. Both these promoters require activation in order to lhnction.

85. How much do you know the transposable virus Mu?
Mu is a mutator phage, which has the unusual property of replicating as a movable genetic element (a piece of DNA acts as transposable element) and can cause the inactivation of the gene it has inserted into. It can be used to generate a wide variety of bacterial mutants very easily.Mu is a kind of virus with large double-stranded DNA which is 39 KB long. But only 37.2 KB is the

actual Mu genome, the rest is host DNA (50-150 base pairs at the left, 1-2 KB at the right end). The head of Mu can be filled with foreign DNA and it brings the foreign genes to other host cells (transduction). It contributes to genetic variation.

86. List the DNA and RNA animal viruses you know.

- Positive-Strand RNA viruses
  - ✓ Picornaviruses, 小 RNA 病毒，+ssRNA 直接用作 mRNA 参与翻译，产生多蛋白联合的蛋白。经过自剪切产生四个结构蛋白、RNA-linked VPg 蛋白，RNA polymerase 用于合成-ssRNA,以及用于剪切的蛋白酶。
- Negative-Strand RNA viruses
  - ✓ Rhabdoviruses, 棒状病毒，包括狂犬病毒 (rabies virus)。外被结构复杂的 envelope, 毒粒内含 RNA-dependent RNA polymerase, 用于合成 mRNA。
  - ✓ Orthomyxoviruses，正黏病毒，包括流感病毒(influenza virus)。外被 envelope,形态不定。流感病毒离体通过 budding,故具有多态性。
- Double-Stranded RNA viruses
  - ✓ Reoviruses，呼吸道肠道病毒。虽然含有+RNA, 但转录过程起始于-RNA 为模板合成 mRNA。最终产生的+ssRNA 分子聚集，并作为模板形成 dsRNA，与蛋白进行组装
- Double-Stranded DNA viruses。
  - ✓ Polyomaviruses，多瘤病毒，有致癌作用。SV40 为其代表种，是向真核生物中转化基因过程中被首先采用的载体，无被膜，二十面体结构。SV 的基因组可被 incorporated into the host genome. 通过转录形成的 mRNA 进入胞质，翻译为 transforming protein 或 T-antigen,后两者有致癌作用。
  - ✓ Herpesviruses, 疱疹病毒
  - ✓ Pox Viruses，痘病毒，包括天花病毒(smallpox)
  - ✓ Adnenoviruses,腺病毒，转录过程中不涉及 lagging strand 上产生的不连续片段，而是通过末端配对的重复片段将 minus template strand 两端粘结起来形成环状 mRNA 分子，然后借助于 virus 编码的蛋白引物和 RNA 聚合酶将进行转录。
- Viruses using transcriptase
  - ✓ Retroviruses，反转录病毒,包括 HIV。含有 RNA genome，以 DNA 为媒介进行转录
  - ✓ Hepadnaviruses，含有 DNA genome，以 RNA 为媒介进行转录。



87. What are the consequences of virus infection an animal cells?
The consequences are:
(1) transformation of normal cells to tumor cells （致癌作用，SV40）
(2) lytic infection （细胞溶解）
(3) persistent infection （持续，指在一定条件下细胞保持良好的生活状态，并不断复制组装进自身基因组中的病毒基因。在特定条件下病毒被激活，导致病症，如 HIV）
(4) latent infection （潜伏，指感染和发病之间存在一定延滞期，如 HSV）

88. How many ssRNA animal viruses (both plus and minus) do you know?
See the answer to 86.

89. Describe the unique properties of influenza viruses.
Influenza virus has segmented genome. Its genome is segmented into 8 linear single-stranded molecules ranging in size from 890-2341 nucleotides, and the segmented genome of influenza virus helps it and other virus of this family exhibit a phenomenon called antigenic shift, in which pieces of the RNA genome from 2 genetically distinct strains that have infected the same cell become reassorted, which result in a change in the surface antigens of the virus. Influenza virus is polymorphic because it buds as it leaves the cell.

90. How do retroviruses replicate themself? What are their uniquenesses? What functions does reverse transcriptase have?
The retroviruses replicate themselves in the following steps:
- Entrance into the cell.
- Reverse transcription of one of the two RNA genomes into a

single-stranded DNA that is subsequently converted to a linear double-stranded DNA by reverse transcriptase.
- Integration of the DNA copy into the host genome.
- Transcription of the viral DNA, leading to the formation of viral mRNAs and progeny viral RNA.
- Encapsidation of the viral RNA into nucleocapsids in the cytoplasm.
- Budding of the enveloped virions at the cytoplasmic membrane and release from the cell.

The unique places are :
- They are RNA viruses, but they replicate by means of a DNA intermediate.
- They were the first viruses shown to cause cancer and have been studied most extensively for their carcinogenic characteristics.
- One retrovirus can cause AIDS.
- The retrovirus genome is unique to have two identical single-stranded RNA molecues of plus complementarity, each 8.5-9.5 kb in length, and 5'-capped and 3'-polyadenylated.
- The retrovirus genome can become specifically integrated into the host genome by way of the DNA intermediate.

The functions of transcriptase are :
- Synthesis of DNA with a RNA template.
- Synthesis of DNA with a DNA template.
- Ribonuclease H activity: an activity that degrades the RNA strand of an RNA :DNA hybrid .

91. How +, - strand RNA viruses replicate? How +, -, or ds strand DNA viruses replicate?
- +strand RNA is the plus strand and can serve directly as a single mRNA. This Mrna encodes the virus specific RNA polymerase, (RNA-dependent RNA polymerase). This polymerase first makes complementary -strands and then uses them as template to make more +strands.
- -strand RNA has to be transcribed into a complementary +strand, then the +strand can be used as mRNA.
- Both +strand DNA and -strand DNA can serve as the template for mRNA synthesis.

92. What are viroids and prions? Describe whatever you know about these two unique viruses.

Viroids 是 small circular ssRNA pathogenic molecules,其胞外形态为 naked RNA, 不含 capsid 蛋白而且 viroids 基因组本身不编码任何蛋白质。其 RNA 复制在宿主细胞的核内完成，类似于 DNA，由 RNA polymerase 合成。

Prions 是只含有蛋白质，不含核酸的感染性病原体，可导致 BSE，CJD(in human)等。编码 prion 的基因位于宿主基因组内，正常蛋白在感染的 misfolded 蛋白和

分子伴侣的作用下发生 misfold。产生的蛋白多在 neuron 中聚集。
注：
目前对 Prion 的研究中多用 Yeast 做模型。PSI+致病，PSI- 正常。涉及的蛋白是 sup35，本来的功能为精确化蛋白合成，在 PSI+中被错误折叠。

6. Microbial Genetics

93. Describe as much as you know about plasmids.

- Plasmids are circular genetic elements that reproduce autonomously and have an extra -chromosomal existence, but no extracellular form.（自主复制，染色体外存在，非胞外存在）
- Naturally occurring plasmids are 1-1000KB in size .Typical plasmid is 1/20 less than that of chromosome. （大小约为染色体长度的 1/20）
- Most plasmids are circular double-stranded DNA, some are linear ds DNA .（多为环状 dsDNA，也有线性 dsDNA）
- They are transmitted from cell to cell via conjugation process.（通过 conjugation 实现胞间传播）
- Most plasmids of gram-positive bacteria replicate by a rolling circle mechanism.
- The copy number of plasmids in a cell can range from only 1-3 copies to 100 copies.
- Closely related plasmids can not both replicate in the same cell.
- Some plasmids can be integrated into a chromosome, and their replication become under control of the chromosome.
- Most plasmids can carry a variety of genes for production of toxin, resistance to antibiotics and heavy metals .et .al.

94. What is the difference between a plasmid and an episome?
Plasmid includes different types; episomes are plasmids having the ability to integrate into host chromosome.

95. What are Hfr strain? F+ or F-, or F' strain?

Hfr strains: Bacterial strains that possess a chromosome-intergraded F plasmid, and show such extensive genetic recombination are called Hfr (for high frequency of recombination)

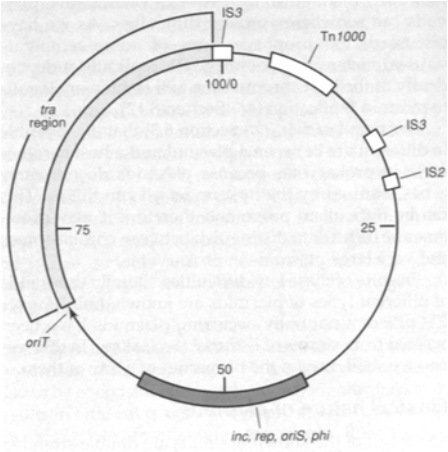
F+ strains: cells possessing an unintegrated F plasmid.

F- strains: cells which can act as recipients for F+ or Hfr, F-strains lack FPlasmid.

F' strains: cells contain F' Plasmids-F plasmids containing chromosomal genes.

96. Draw the F plasmid and describe functions of various DNA regions.

inc: incompatibility
oris: Origin of replication
Tn: Transposable elements
tra region: transfer functions
phi: phage inhibition
IS: insertion sequence
rep: replication functions
oriT: origin of transfer



97. Why is it said that conjugative plasmid contributes to evolution?

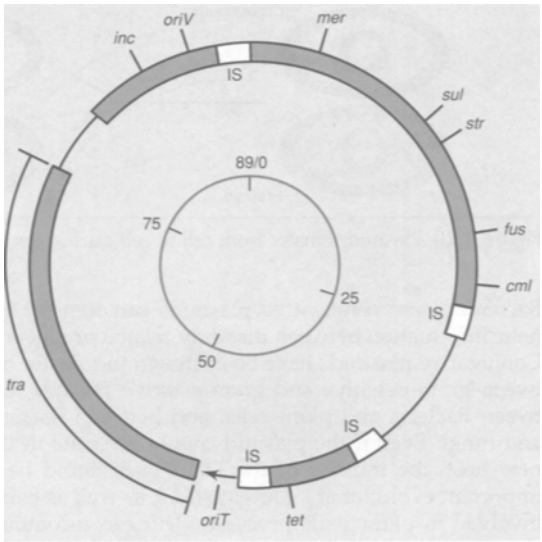
Some conjugative plasmids can transfer genetic information between distinctly related organisms (between gram-negative and gram-positive Bacteria, between Bacteria and plant cells; and between Bacteria and fungi). Even if the plasmid cannot replicate in the new host, the transfer of tile DNA itself could have important evolutionary consequences if it can recombine into the genome of the new host.

98. How many types of plasmids and their functions you have learned?

(1)Conjugative plasmid- enables the microorganism the ability of conjugation.
(2). R plasmid----enable the microorganism to resist to a wide variety of antibiotics and various other inhibitors of growth.
(3). Bacteriocin----inhibit or kill closely-related species or even different strains of the same species.
(4). Virulence plasmid----enable the microorganism to attach to and colonize specific sites in the host and form substances (toxins, enzymes, and other molecules) that cause damage to the host,

99. Schematically describe R100 plasmid and its functions.

Inc, incompatibility genes
oriV: origin of replication site
oriT origin of conjugative transfer
mer, mercuric ion resistance
Sul ,sulfonamide resistance
tet, tetracycline resistance
tra , transfer functions
IS , insertion sequences



100. How do R plasmids inactivate antibiotics?

Because a single R plasmid contains a variety of genes coding for different antibiotic inactivation enzymes.

101. What is an engineered plasmid?

Engineered plasmids are artificial plasmids that have been incorporated of genes from a wide variety of sources to made possible the transfer of genetic material across any species barrier or be synthesized completely from new genes. They are important tools in understanding of the structure and functions of plasmids.

102. What points do F plasmid provide to its host?

F plasmids provide to its host cell with:
(I). ability to synthesize the F pilus
(2). mobilization of DNA for transfer to another cell
(3). alteration of surface receptors so that the cell is no longer able to act as receptors in conjugation.

103. How to detect genetic recombination. Please cite one example.

The cell resulting from recombinant must be phenotypically different from the parents to be detected. The recipient stains usually used lack some selectable characteristic that the recombinants will possess. For example, drug resistance. Then we can detect the recombinants by cultivation in the presence of sufficient concentration of antibiotics.

104. How is bacterial genome mapped? What are the three types of transposable elements?

Interrupted Mating is used in bacteria genome mapped. First mix Hfr and F cells. Then shake the mixture violently at various times and plate the samples on selective medium for recombinant to grow. At last measure the percentage of the recombinants in the bacteria. The longer the time between pairing and agitation, the greater the number of genes of the Hfr that will appear in the F recombinant. Genes present closer to the origin enter the F first and are always present in a higher percentage of the recombinants than genes that enter late. In addition, gene transfer from donor to recipient is a sequential process. This can show the sequence of the genes.

Three types of transposable elements:
(I). Insertion sequence (IS): about 1-1000 nucleotides, carry only information to move to new location. (IS1, IS2, IS3)
(2). Transposons (Tn): larger than IS, carry genes, such as drug resistance markers and other selectable genes, can move from one location on a bacterial genome to another and from one bacteria to another.
(3). Some special viruses (such as Mu)

105. Explain transposon mutagenesis and its possible application.

If the insertion site for a transposable element is within a gene, insertion of the transposon will result in loss of linear continuity of the gene, leading to mutation, termed transposon mutagenesis.

Transposon with antibiotic-resistant marker can be used for selection purposes.

106. Give an example to explain conversible DNA and phase variation.

Each cell of the enteric bacteria *Salmonella* has two genes，Hl and H2, coding for two different flagellar proteins, but only one of the two genes is expressed at any one time, Invertible element is involved in expression of the flagellar proteins. When the invertible segment is in one orientation, the H2 gene is transcribed, but in addition, another gene transcribed that codes for a protein that represses transcription of gene Hl. Thus, when H2 is expressed, Hl is turned off. On the other hand, when the invertible segment is in the opposite orientation, the genes for H2 and the H1 repressor are no longer expressed, so H1 can now be transcribed.

107. What techniques are used to map bacterial DNA genome?

The three methods of genetic exchange (Transformation Transduction and Conjugation) are used to map the location of various genes on the chromosome.

Conjugation: map the particular region of the chromosome

Transduction: map more fine structure of the chromosome

7. Industrial Microbiology

108. What major industrial products have been produced by microbial fermentation processes?

- Microbial cells--yeast cultivated for baking, food, brewing, mushrooms cultivated for food （微生物活体细胞）
- Enzymes--glucose isomerase, lippases, proteases, renin, amylases（具体外活性的功能酶）
- Pharmaceutical agent--antibiotic, steroids, alkaloids（药物组分）
- Food additives--amino acids, vitamins（食品添加剂）
- Special chemical and food additives: aspartic acid, phenylalanine （专用化合物及食品添加剂）

109. What steps are required for the development of an industrial microbial fermentation process?

- Strain selection (纯化菌株选取)
- laboratory process development （实验室培养）

- pilot scale up （小规模发酵）
- industrial scale up （工业化发酵）
- downstream process development （下游产物加工）
- product packaging technique （产品装配技术）
- other commercial consideration （其他经济因素考虑）

110. How to obtain a strain for our fermentation process development?

We can obtain a strain for fermentation process development from:

- Purchase from Culture Collections （直接购买）
- Screening of nature circumstances （从自然界筛选）
- Genetic engineering （基因改造）
- Mutations（基因突变）
- Cell biology technique （细胞技术）

111. How to carry out a lab scale fermentation process?

We have 4 type lab scale fermentation process:

- Batch process: All the nutrients needed for cell growth will only be added once at the beginning of fermentation.（一次接种，平台期取料）
- fed-batch process: During the fermentation, additional nutrients will be added in a batch way to promote the cell growth or product formation and to avoid nutrient deficiency （一次接种，部分取料后加入新的机制继续发酵。）
- semi-continuous process: Similar to continuous process, with the addition of nutrients and outflow of fermentation broth in a continuous and batch way.
- continuous process: （定速给料，并回收产物和过量基质，一般不用于人消费用产品的生产因为无菌性难以保证。）

具体的操作过程包括：

- 选择优良菌株
- 优化菌株生长和产物生成条件
- 优化培养基质
- 动态研究，确定四个生长时期以计算最佳的取料时间和获得最大量的产品

112.Why do we need to use shake flasks for lab scale process development? What can we obtain from shake flask experiment?

Because we have to make sure whether the strain is suitable or not for scaling up. We have to find several (optimal) conditions and improve the product. To reduce the cost , if we fail in the lab we can change the cultivation conditions , strain , environment factors without wasting too much . We can obtain the optimal conditions such as the suitable strain, environment factors (temperature, pressure, OD etc). to procedure the product

Optimization of conditions for cell growth and product formation using shake flask experiments:

- pH

- Temperature
- Dissolved oxygen (DO)
- Substrate choice
- Maximal and optimal substrate concentration
- Others

113. What is scale up? Why do we need a pilot experiment before we move to industrial scale production?

Scale up: conversion of an industrial process from a small laboratory setup to a large commercial fermentation.

We do pilot scaling up because the cost is less then commercial scale and the conditions are more closely resemble as the commercial scale. We can use the instrumentation and computer control, further more if we fail we can change the conditions, find out the problem and solve it more easily and faster than commercial scale.

注：

不断重复的 scale up，实验室内的试验和工厂化前期试验都是为了一步步模拟实际的工厂化发酵生产的真实条件，并在实验过程中不断摸索菌中的生长特性、最适条件、投料取料的最佳时间以及可能出现的问题等等因素。同时，为了避免最终工厂化生产中出现失败导致重大损失，初期的试验及改进无疑是必要的。

115.What parameters must be monitored during fermentation?

- The concentration of the product, substrate, enzymes (基质、产物和酶的浓度)
- Control of other microorganisms, (无菌技术，其它微生物生长的控制)
- environment factor such as OD, pressure , temperature, agitation rate for fermentation etc.（最适环境因素的保证）
- Substrate consumption, product formation, cell dried weight.（基质消耗，产品产出以及细胞干重于发酵时间的关系以确定投料、取料时间）

In most cases, it is necessary not only to measure growth and product formation but also to control the process by altering environmental parameters as the process proceeds. Environmental factors that are frequently monitored include temperature, oxygen concentration, pH, cell mass, and product concentration.

116.Schematically describe a fermentor.

Fermentor is the vessel in which the industrial process is carried out. It is VARIABLE in size and are depend on the process and how it WILL BE USED. It must have pH controller, impeller, motor, sterile seal, sparger, cooling jacket, culture broth, harvest OUTLETS, cooling water out and in.

117. What is the most critical problem during process scale up in microbial fermentation?

The most critical problem is surface-volume ratio decreases in bigger vessels, which caused decreased oxygen gas transfer efficiency.

随着培养基和培养容器体积的增大，其表面积/体积之比在不断缩小，导致内部物质循环的效率不断下降。最重要的体现在氧气的传输效率显著下降，需要引入机械搅拌和加大氧气输入来解决问题。

118.How to maintain a constant dissolved oxygen concentration during the fermenation process?

We can contain it with

- Increase stirring rate, 加快搅拌速度
- increase air pressure, 增大氧气压强
- use pure oxygen, 使用纯氧
- increase air inlet. 增加空气输入量

119. How to make high-fructose corn syrup?

There are 3 steps and 3 enzymes.

The enzymes include:

- α-amylase: brings about the initial attack on the starch polysaccharide, shortens the chain, and reduces the viscosiy of the polymer. (thinning reaction)消化淀粉为多糖
- Qlucoamylase:produces glucose monomers from the shortened polysaccharides, namely ologosaccharide.(saccharification)消化多糖为葡萄糖
- glucose isomerase: glucose convert to fructose.(isomerization) 葡萄糖异构酶将葡萄糖转变为果糖

120. How many ways can you use to immobilize enzymes or cells?

We can immobilize enzymes or cells in 3 ways :

- cross-linkage (polymerization) of enzyme molecules.
  Usually done by chemical reaction with a bifunctional cross-linking agent. involve the amino groups of the enzyme protein with cross-linking agent like glutaraldehyde. 交联
- Binding of the enzyme to a carrier.
  Through adsorption, ionic bonding, or covalent binding.载体
- Enzyme inclusion
  Involve incorporation of the enzyme into semi-permeable membrane.
  Enzymes can be enclosed inside microcapsules, gels, semi-permeable polymer membranes, or fibrous polymers. 包被

121.Do you know how vinegar, citric acid, yeast are made?

Vinegar(醋)

the product from the conversion of ethyl alcohol to acetic acid by acetic acid bacteria. 酒精通过醋酸菌转变为醋酸

Materials: wine or alcoholic apple juice(cider), mixture of pure alcohol in water(distilled vinegar).acetic acid bacteria.

Key requirement of vinegar production is sufficient oxygen supply duting the fermentation because acetic bacteria are high oxygen demanding during growth.

Three type process :

- Open-vat (Orleans method)
  Wine is placed in shallow vats with considerable exposure to the air, acetic acid bacteria develop as a slimy layer on the top of the liquid. The method is not very efficient. Only the substrate in the surface is product to vinegar.
- Trickle method
  Trickle the alcoholic liquid over beechwood twings or wood shavstream of air enters at the bottom and passes upward. It is operated in a continuous fashion.
- Bubble method
  Alcohol liquid is added at a rate just sufficient to balance removal of vinegar. The product must undergo more filtering to remove the bacteria.

Citric acid(柠檬酸)

primary metabolic product formed in the tricarboxylic acid cycle.

Key requirement: medium must be iron deficient and aerated.

Material: carbohydrate sources (starch, glucose sucrose , several kind of sugar).fungus.

Two type product process:

Surface processes: culture medium is solid or liquid, solid substrates. Take 5-8 days.

Submerged processes: apply 3 factor-(a)quality of the material used to construct the fermentor.(b)mycelium structure.(c)oxygen supply.

Yeast:

key requirement: can't added all the molasses（糖蜜） to the fermentor at once The yeast will convert some sugar to alcohol.

Material: molasses, phosphoric acid, ammonium sulfate.

Process：

- pure stock culture
- several intermediate stages (flask culture, seed culture, intermediate fermentor) needed to build up the inoculum to a size sufficient to inoculate the final stage(production fermentor)
- recovered from the broth by centrifugation
- washed by dilution with water and recentrifuged until they are light in color

- package.

122. Describe how the following alcoholic beverages are made: wine, dry wine, sweet wine, fortified wine, sparkling wine, whiskey, brandy, rum, vodka?
sparkling wine, whiskey, brandy, rum, vodka?

There are only last part is different from wine , dry wine , sweet wine , fortified wine, sparkling wine, whiskey , brandy, rum , vodka have been made. They are all fermated by yeast just different in the alcohol concentration , the sugar left in the product and the last fermentation occur. Dry wine r wine in which the sugars of the juice r practically all fermented, sweet wines r still some sugar left or additional sugar is added after the fermentation; whiskey , brandy, rum, vodka : the distilled alcohol is added after fermentation; Fortified wine is one to which brandy or some other alcoholic spirit is added after the fermentation; sparkling wine is one in which considerable CO2 is present, arising from a final fermentation by the yeast directly in the bottle(secondary fermentation).

The main process to make the wine are like below:

- harvesting of grapes
- crushed the grapes-> must
- adding SO2 to kill the wild yeast
- press
- fermentation
- filtration(racking)

123. How mushroom is grown?

Cultivated in mushroom farms. The organism is grown in special beds (prepared by mixing soil with a material very rich in organic matter), in buildings where temperature and humidity are carefully controlled. If light is not necessary, they can be grown in basements of homes, in caves. Beds are inoculated with mushroom spawn (pure culture of the mushroom fungus that grown in large bottle).
When mycelium grows and spreads through the substrate in bed, add to the sruface of the bed a layer of soil(casing soil) and when flushing occurs the mycelium is collected immediately. Finally they are packaged and kept cool.

124. What is the most critical problem during process scale up in microbial fermentation?

125. How to maintain a constant dissolved oxygen concentration during the fermenation process?

126.What are the strengths and weaknesses of a technological process.

Strengths
1. reliance on renewable feedstocks
2. versatility with different feedstocks
3. food,feed &drug application
4. fine chemicals to bulk chemical applications
5. low temperature
6. operation in aqueous media
7. several reactions achieved in a single fermentation step
8 .high level of automation
9. stereols specificity
10. complex molecules converted & or produced
11.'Benign' effluents producted

Weaknesses:
1. feedstocks are oxidized & unsuitable for many application
2. feedstock costs fluctuate
3. sterilization is a major cost
4. product often in dilute aqueous solutions
5. product recovery costly
6. equipment costs high
7. reactions slow leading to poor volumetric productivity
8. complicated reaction conditions
9. mainly batch operation
10. high cell(catalyse) regeneration costs
11 .high BOD wastes.

127.How to develop a fermentation process for antibiotic production?

isolation or collection of cultureses->
screening of cultures to detect those with antimicrobial activity->
development of methods for submerged-culture production->
development of methods for isolation & purification of antibiotic->
determination of antibiotic properties->
(physical: adsorption & absorption
chemical: reactions, solubility in solvents ,stability to acids, alkalis, heat etc.)
evaluation of antibiotic->
pharmacological tests->
antimictrobial activity->
comparison with existing antibiotic->
development of pilot plant production methods->
submission of licence for clinical trials->
testing of purified antibiotic->
development of plant scale production methods->

obtaining a product licence for clinical use->
other miscellaneous considerations:
development of methods to control production of antibiotic
development of new applications
development of marketing & distribution system
financing of business

128.What will you do if you are assigned as a chief engineer to develop a fermentation-based project?
先考虑以下问题：
1)市场需求
2)目前关于这方面的资料&所要开发的产品的情况。
3)赞助经费来源&夺得资助费的可能性
4)如何取得好的菌种(find the new strain)
5)产品的用途，优缺点，是否会引起环境污染 etc
6)所须的原料来源
7)收率
进行以下步骤：
(一)实验阶段(laboratory process development)
1)选取适合的育苗：通过各种各样的试验手段选取，改良(增强物理化学稳定性，降低毒等)，筛选适合的育苗
2)提纯所须的育苗或基因片段
3)在实验室进行试验以夺得相关的条件，如环境条件(T ，P，PH ，OD....)，需要改进的地方
4)重复 3)以夺得相关的，所须的资料
5)复制所须的育苗或基因片段
6)pilot scale up
(二)寻找投资者
(三)扩大生产(industrial scale up, downstream process development)
(四)经各方面的检测&手续处理
(五)出售: includes product packaging techniques

129．What are the concepts of genetic engineering and gene cloning?
Genetic engineering: isolation, manipulation, and expression of genetic materials.
Gene cloning: isolation and incorporation of a gene into a vector where it can be replicated(purification of specific genes).

130．Describe the gene cloning procedure.
1)isolation and fragmentation of the source DNA
2)joining the DNA fragments to a cloning vector with DNA ligase
3)incorporation into a host
4)detection and purification of the desired clone

5)production of the large numbers of the cells or bacteriophage containing the desired clone for isolation and study of the clone DNA

131. Schematically describe the Plasmid pBR322.
1)small, only 436 kbp
2)stable in E.coli
3)high copy number(1000-3000copies/cell) >10kilobases make it instability
4)easily isolated in the supercoiled form by simple techniques
5)foreign DNA can be inserted in good amount
6)restriction site are known
7)single cleavage sites for several restriction enzymes
8)2 antibiotic resistance markers: ampicillin and tetracycline.
9)transformation easy

132. How to clone and express a foreign gene in pBR322?
1)used BamHI or PstI to digest the foreign DNA to fragments and pBR322
2)joining the DNA fragments to a pBR322 with DNA ligase: the DNA fragments will be inserted into the plasmid.
3)introduction of the vector into E.coli by transformation.
4)if the foreign DNA is inserted into the plasmid, the E.coli will just be resistant to ampicillin else tetracycline. those who resistant to both are lack the foreign DNA. So we can detect them.

1）用 BamHI 或 PstI 把 pBR322 和外源 DNA 切开
2）用 DNA 连接酶将外源 DNA 片段连接到 pBR322 的特定部位
3）用转化技术将重组 pBR322 导入 E.coli
4）有外源 DNA 的质粒只对一种抗生素有抗性，由此可以检测出插入成功的质粒

133. How to use a modified lambda phage as cloning vector?
1) isolation of the vector DNA from phage particles and digestion with the appropriate restriction enzyme.(从 phage 中分离出载体 DNA，用适当的限制煤切割。)
2) connection fo the 2 lambda fragments to fragments of foreign DNA using DNA ligase. Conditions are chosen so that DNA molecules have a length suitable for packaging into phage particles.(用 DNA 连接酶将外源的)
3) packaging of the DNA by adding cell extracts containing the head and tail proteins and allowing the formation of viable phage particles.(加入 phage 头和尾蛋白，体外包装，形成完整的噬菌体颗粒)
4) infection of E.coli and isolation of phage clones by picking plagues on a host strain.(感染 E.coli，通过挑选噬菌斑而分离噬菌体克隆)
5) checking recombinant phage for the presence of the desired foreign DNA

sequence by nucleic acid hybridization procedures or observation of genetic properties.(利用核酸杂交或遗传性状检测选出携带外源 DNA 的重组 phage)

134. What is a cosmid? a YAC? an expression vector and secretion vector? a shuttle vector?

Cosmid: plasmid vectors containing foreign DNA plus only one cos (cohesive end) site from the lambda genome.
携带外源性 DNA 的质粒加上 lambda phage 的 cos(粘性末端)位点构成。
YAC(Yeast artificial chromosomes): the vector which have an origin of DNA replication, telomeres at the ends of the chromosome, a cloning site and a gene that can be used for selection after transformation into the host. Can have 200-800 kbp of cloned DNA inserted. Usually used to cloning the eukaryotic DNA, especially for the human genome project.(p 366)
Expression vector: a vector which not only can be used to clone the desired gene but also contains the necessary regulatory sequences so that expression of the gene is kept under control of the genetic engineer.(to obtain synthesis of the protein coded for by the foreign gene cloned into the vector)
Secretion vector: which the protein product is not only expressed but secreted (excreted) out of the cell.
Shuttle vector: the vector that can replicate in two different organisms so is used to move DNA between unrelated organisms.

135. What properties an organism should have in order to be used as a host for expressing foreign genes?

1) rapid growth.
2) capable of growth in cheap culture medium.
3) not harmful or pathogenic.
4) transformable by DNA.
5) stable in culture.

136. How many prokatyotic and eukaryotic hosts for expressing foreign genes you have known?

Prokaryotic host: E.coli/ Bacillus subtilis(枯草芽孢杆菌)
Eukaryotic host: Saccharomyces cerevlsiae(yeast)(面包酵母) / insect cell line(昆虫细胞)

137. What vector can carry the largest foreign genes? the smallest? (YAC, Plasmids, Lambda, Cosmid)

YAC>cosmid>lambda>plasmids

138. What is a phagemid?

A hybrids between a filamentous phage, like M13, and a plasmid

.

139. What are the methods to find the right clones?

1) method for detecting production of protein by use of specific antibody(in radiolabled form).(用特定抗体检测蛋白)
2) Detecting production of protein by its activity.(检测蛋白的活性)
3) Method for detecting recombinant clones by colony hybridization with a radioactive nucleic acid probe.(与放射性核酸探针杂交检测重组克隆)
4)

140. Describe PCR method

PCR(polymerase chain reaction) is a unique procedure for amplifying DNA in vitro, makes use of a heat stable DNA polymerase from thermophilic prokaryotes. Heat is used to denature the DNA into 2 single-stranded molecules, each of which is copied by the polymerase. Beginning with a small oligonucleotide that serves as a primer for the target DNA to be amplified, the polymerase copies the complete DNA to which the primer associates. After a single copy cycle, the newly formed double strands are separated by heat again, and a new round of copying permitted. At each thermal cycle, the amount of target DNA doubles. Under appropriate conditions, a billion-fold increase in the target DNA can be obtained.

利用可耐高温的 DNA 聚合酶和一个小的寡核苷酸为引物，以 DNA 在高温时会变性成两条链及降温后又可复性的原理，将 DNA 在引物的作用下，当变形时以两条 DNA 链为模板由 DNA 聚合酶复制所要得 DNA。第一次循环后，每当高温时就以复制所得的 DNA 链为模板复制，退火时复制结束，完成一次循环。每经一次循环作为模板的 DNA 就增加，以指数倍增加，使 DNA 得以大量复制。

141. What is the principle behind DNA fingerprint?

DNA fingerprinting:

used of the techniques of genetic engineering to determine the origin of DNA in a sample of tissue.

the principle of DNA fingerprint:

the higher eukaryotes contain a vary large amount of repetitive DNA and have VNTRs(variable number of tandem repeats) which is different in the number of repeats in every individual alleles. 2 different alleles of a region of a single chromosome only differ in the number of repeats in the VNTR. Since the VNTR DNA has been sequenced, it is known what restriction enzyme site are not found in a particular VNTR. Digestion with such an enzyme then releases the complete VNTR intact. When the DNA from 2 chromosomes with a different number of repeats at this particular locus is digested, the restriction fragments containing this DNA will differ in size. It can be separated by gel electrophoresis, and the VNTR-containing fragments detected by Southern blotting using a probe made from the cloned VNTR sequence.

高等真核生物的染色体中含有重复的 DNA，其中有一些重复序列在不同的个体中具有不同的重复次数，成为 VNTR，此序列可被特定的酶切割，故在不同的个体的等位基因中所含的 VNTR 的重复次数不同，所以将被切割成不同大小的片断，可由此用凝胶电泳或 Southern blotting 的方法鉴定是哪个个体的 DNA。

142. Describe cloning and expression of mammalian genes in bacteria using mRNA route and protein route.

1）mRNA route:

在细胞中原有 1%-5%的 mRNA，利用 mRNA 具有 polyA 尾的特性，将富含 polyA 的 RNA 通过含有 polyT 片段的层析柱，由于 A、T 碱基对之间的相互作用，使得细胞中大部分 mRNA 分离出来。

Protein route:

通过 reverse translation, polyribosome precipitation 合成 DNA。

Reverse translation:

事先分析出蛋白质的氨基酸序列，依照三连密码子一个氨基酸的原理，在以各不同生物的不同密码子反翻译可能的 mRNA。以此 mRNA 合成与其部分上互补的 DNA。此 DNA 作为 Northern blot 的探针进行杂交获得目的 mRNA。

Polyribosome precipitation:

将细胞中合成蛋白的核糖联合体（ribosome complexes）分离出来，用抗体与所要的蛋白结合，从而分离出带目的 mRNA 的核糖体联合体。

2)将目的 mRNA 以 oligo-dT primer 为引物，在反转录酶（reverse transcriptase）的作用下，转录成 cDNA。

3）将 cDNA 连接到载体上，形成重组 DNA 分子。

4）转化到寄主中，在寄主中形成寄主的重组 DNA 分子。

5）进行配用，大量复制后，表达出产物蛋白。

143. How to carry out the in vitro and site-directed mutagenesis?

1） 将源基因（source gene）克隆到单链的载体，一般用 M13 的单链 DNA 为载体（M13ssDNA）。

2） 加入人工合成的含有一个不配对的碱基的 oligonucleotide (synthetic oligonucleotide with one base mismatch)，其序列已知。

3） 用 DNA 聚合酶（DNA polymerase）延长此单链以复制剩余的 DNA 序列，形成双链 DNA。

4） 将此双链 DNA 分子以转化（transformation）的方式插入到寄主中克隆。

5） 筛选出突变体。

144. How to disrupt a gene using cassette mutagenesis?

当基因被任意的酶的切点很靠近则可将介于其间的 DNA(intervening DNA)切除用合成的 DNA 片段代替，此合成的 DNA 片段即是 cassettes。

一般用酶，如 EcoRI 酶切包含在 plasmid 的野生型目标基因并将其与 DNA 片段连接。此时，plasmid 具有含卡那霉素的突变型基因。将此 plasmid 用另一种酶如 BamHI 切割成单链的 plasmid 并转化到该野生型基因的细胞中。由于同源性（homologous）将使同源细胞中的染色体中的野生型基因与 plasmid 中突变型基因发生同源性的转化（homologous recombination）使该基因失去功能。所有可在含卡那霉素生长的细胞即是突变体。

145. How many products have been produced using genetic engineering techniques?

1) antibiotics, enzymes, proteins, biopolymers etc.
2) virus vaccines: expression of virus protein coats.
3) Mammalian protein: like hormones--insulin for treating diabetics, human growth hormone for treating dwarfism, epidermal growth factor for stimulating wound healing, bone growth factors for treating osteoporosis, animal growth hormone for stimulating growth of livestock animals.

146. Describe how to carry out plant genetic engineering.

We need binary vector, which containing ends of T-DNA, foreign DNA, origoin of replication elements for both E.coli and Agrobacterium tumefacens, and spectinomycin and kanamycin resistance markers(can be selected for in plants). First ,we put the binary vector into E.coli to cloning the foreign DNA , then transferred the vector to Agrobacterium tumefacens's Ti plasmid by conjugation. The foreign DNA to be transferred must be between the ends of the T-DNA(the segment of the Ti plasmid DNA that is actually transferred to the plant). Final transfer to the plant depends on the Agrobacterium tumefaciens. D-Ti can mobilize the T-DNA region of the vector for transfer to plant cells grown in tissue culture. From the recombinant plant cell, whole plants can be regenerated.

1）把二元载体植入 E.coli 去克隆外源 DNA
2）把此载体转入根癌农杆菌的 Ti 质粒（通过接合）
3）靠根癌农杆菌把 DNA 转入植物

9. Microbial ecology

147. What is Microbial Ecology and What do microbial ecologists study?

Microbial Ecology refers to the microorganisms in soil, water, and other environments and how microorganisms act to chemically change their environments.

Microbial Ecologists study:

a. the biodiversity of microorganisms in nature and how different guilds interact in microbial communities.
b. The activities of microorganisms in nature and monitor their effects on ecosystems.

生态学是一门研究生物系统与环境条件间相互作用规律性的科学。微生物生态学就是研究微生物群体----微生物区系（microflora）或正常菌群（normal flora）对其周围的生物和非生物环境条件相互作用关系的科学。微生物生态学家就是要弄清自然界中微生物的多样性和在微生物群落中不同的共位群间的相互作用，测定自然界中微生物的活动并且监测他们对生态系统的影响。

148. What is a microevironment?

The immediate environmental surroundings of a microbial cell or group of cell.
指微生物细胞或细胞群周围的环境，包括其周围小生境中的不同资源类型、数量以及生理化学条件（温度、pH、湿度、光照、氧等）

149. Describe biofilms and their functions and significance in practical applications.

Biofilms are encased microcolonies of bacterial cells, attached to a surface by way of adhesive polysaccharides excreted by the cells.
They can trap nutrients for the growth of the encased microbial population and help prevent detachment of cells on surfaces in flowing systems.
Significance:

a. in the human body, bacterial cells within a biofilm are made unavailable for attack by the immune system.
b. Dental plague, a typical biofilm, contains acid-producing bacteria responsible for dental caries.
c. In industry, biofilms can slow the flow of water or oil through pipelines, accelerate the corrosion of the pipe themselves.

大多数微生物生长在被生物膜（biofilm）覆盖的表面上，细菌细胞的小菌落附着在表面是由于细胞分泌粘性多糖。生物膜可诱捕营养使附着的微生物生长，可以帮助阻止表面上的细胞在流动的系统中脱离。
1、在人体中，包在生物膜中的细菌细胞可免受免疫系统的攻击，使人造表面的利用变得复杂。
2、医学上移植时，它可作为含有病原体及微生物的生物膜发育部位。齿斑也是典型的生物膜。
3、工业上生物膜使管道液体流速减慢，加速管道腐蚀。

150. What are the factors affecting microbial ecology?

Nutrient level and growth rate, Microbial competition and cooperation and so on.
营养水平，生长速率，微生物的竞争和协同作用。

151. List methods commonly used to study microbial ecology.

Study biodiversity: isolation; identification and quantification of microorganisms in various habitats.
Study microbial activity: radioisotopes, microelectrodes.
研究微生物多样性的方法包括不同环境中微生物的分离（富集、纯培养）、鉴定和定量（核酸探针、荧光抗体、活菌计数）。
研究微生物活性的方法有放射性同位素法和微电极法。

152. What is the Winogradsky column and the bacteria enrichment technique?

The Winogradsky column: for isolation of purple and green phototrophic bacteria and other anaerobes.
Enrichment culture technique: a medium and a set of incubation conditions are used that are selective for the desired organism and are counterselective for the undesired organisms.

Winogradsky 柱是用来分离紫色和绿色光合营养细菌以及其它厌氧菌的传统

装置。
利用一定的培养基和培养方法选择所需要的生物，而对不需要的生物进行反选择的技术称为富集培养技术。

153. What is the Winogradsky column used for? Schematically draw it.

Winogradsky 柱是用来分离紫色和绿色光合营养细菌以及其它厌氧菌的传统装置。它用于各种原核生物的富集，包括需氧菌和厌氧菌。

154. How to carry out isolation of anaerobic bacteria in pure culture?

The objective of an enrichment culture study is usually to abtain a pure culture. Pure cultures canbe obtained in many ways, but the most frequently employes means are the streak plate, the agar shake, and liquid dilution methods. The agar shake method is most useful for oxygen-sensitive anaerobes. It involves the dilution of a mixed culture in tubes of molten agar, resulting in colonies embedded in the agar rather than on the surface of a plate.

最经常使用的纯培养方法主要有：划线平板法、琼脂振荡法和液体稀释法。分离厌氧菌要考虑到氧气的影响，尽量使细菌与氧气隔离开。所以琼脂振荡法比较好。它是在含熔化的琼脂试管中混合不同稀释浓度的培养物，使产生的菌落埋在琼脂中而不是在平板的表面，这就有效隔离了空气。纯化也可以在含有液体培养基的试管中连续稀释细胞悬浮也，达到最适浓度，不过也要注意隔绝空气。

155.How to do Identification and Quantification of environmental microorganisms using Nucleic Acid Probes, Fluorescent Antibodies, and Viable Counts?

- 核酸探针：利用核酸探针与 16s 的互补性，某一特定的核酸探针将会与特定种群的 RNA 结合,并通过放射性同位素或者荧光材料显示出来。
- 荧光抗体:利用抗体对特定生物细胞表面组分的特异结合并发出荧光，可以鉴定出土壤中特定微生物。
- 培养记数：每一个活的细胞都能够培养出一个群落，我们可以根据微生物在琼脂培养基上培养出的菌落数来确定细胞的数量。

156.How to do the Measurements of Microbial Activity in Nature using radioisotope technique and microelectrodes?

- 放射性同位素法：将碳 14 或者硫 35 等放射性同位素的化合物(适量)与生物共同培养，通过放射性的信息获得微生物的活力信息。
- 微电极法：用特制的微电极测定 PH，O2,N2O,S 等物质浓度或者物理量。方法是：将微电极插入微生物生长的生境中，并在显微操作器的帮助下移动 50-100 微米读取数值。

157.What Stable Isotopes are used in Microbial Biogeochemistry? How to decide the biogenic or abiogenic of a material?

稳定同位素有碳 13 和硫 34。

判断物质是不是生物合成的可以根据生物体的酶对较轻元素的偏好，生物合成的物质含有较环境较高的碳 12。

158. What are aquatic habitats and primary producers? How do they distribute in nature?

水生环境是指:海洋，港湾，盐沼，湖泊，池塘，河川，泉水等等。
初级生产者：利用光能，从 CO2 合成有机物的生物。
初级生产者分布在近海海域，湖泊，泉水中等，而在远海海域则分布较少。

159.What is Biochemical Oxygen Demand (BOD)?

BOD 是环保工作者用来描述水体耗氧属性的一个量。
BOD 的测定：将充分供气后的水样密封在瓶中，培养一定时间（通常为 20 度，5 天），然后测定水中剩余的氧气量，由此即可得到 BOD 数值。

160. How are microbial activities looked like in various layers of soil?

在土壤表层(A)微生物活跃—因为其中含有大量的有机物；
在地图层（B）微生物活动较 A 层为少—其中含有的有机物比较少；
在土壤基质（C）微生物活动较少。

161. What are the conditions under deep sea? What microorganisms inhabit in deep sea?

深海环境特点：低温（1000m,2~3 度）；
高压（1atm/10m);
低营养条件。

深海中的微生物有：barotolerant and barophilic bacterias,as well as obligate barophilic bacterias.

162.What makes microorganisms under deep sea so unique compared with normal microbes?

为了适应深海的低温，高压，低营养条件，深海微生物具有一些特点：酶的折叠程度较高，细胞膜不饱和脂肪酸增加，在蛋白质合成和跨膜运输上也具有特殊性。

163. Why can tube worms live under deep sea where little organic nutrients are available?

管虫有一个改进的肠胃道，是由营养体的海绵组织组成的。营养体组织充满了硫颗粒，并含有大量的原核细胞。营养体组织具有硫氰酸生成酶的活性和三羧酸循环中的酶。管虫营养由化能无机营养细菌提供，是以化能无机营养体分泌物和死的细胞为生。管虫明亮红羽毛富含血管，用独特的可溶性血红蛋白结合 O_2 和 H_2S，作为 O_2 和 H_2S 的捕捉器，运输化能无机营养菌到营养体中。

164.List microorganisms you have known that tend to live in hydrothermal vents?

化能无机营养菌 Sulfur-oxidizing bacteria：硫氧化细菌（硫杆菌属 Thiobacillus、硫微螺菌属 Thiomicrospira、发硫菌 Thiothrix、贝日阿托氏属 Beggiatoa）;硝化细菌 Nitrifying bacteria; 硫酸盐还原细菌 sulfate-reducing bacteria; 产甲烷细菌 Methanogenic Archaea; 氢氧化细菌 Hydrogen-oxidizing bacteria; 铁和锰氧化细菌 Iron and manganese-oxidizing bacteria; 甲基营养细菌 Methylotrophic bacteria。

165. Schematically describe carbon and oxygen cycles.

碳是在整个地球的主要储存库中循环，发生于空气、陆地、海洋和其它水环境沉积物和岩石。地球上最快速的碳转移方式是空气中的 CO_2，主要通过陆地植物的光合作用除去大气中的 CO_2，然后 CO_2 又通过动物和化能有机营养微生物的呼吸作用回到大气中，微生物对死的有机物包括腐殖质的分解作用使 CO_2 回到大气。合成新的有机碳唯一主要方式光合作用（合成新的有机碳唯一主要方式），生氧光合作用总反应：CO2 + H2O→(CH2O) + O2，呼吸作用：(CH2O) + O2→CO2 + H2O。分解作用，降解光合作用固定的碳，甲烷和二氧化碳（两种主要碳氧化作用状态），这两种气体产物是由产甲烷菌或各种化能有机营养菌通过发酵，无氧或有氧呼吸形成的，当甲烷被运输到有氧环境时，产甲烷细菌将其氧化成 CO2，然后所有碳最终回到 CO2，自养代谢又开始。

166. What microorganisms are involved in C and O cycles?

藻类（光合作用）、蓝细菌（光合作用）、微生物（主要化能有机营养微生物，呼吸作用）、化能无机营养菌、甲烷氧化细菌－甲烷营养菌、产甲烷菌、同型乙酸细菌、光合营养细菌、厌氧微生物（发酵）。

167. How is methane produced from complex organic materials via microbial activities?

甲烷产生是由古生菌—产甲烷菌来实现的，大多数在厌氧呼吸中利用 CO2 作为最终电子受体，还原成甲烷，电子供体一般为 H2：4H2+CO2→CH4+ 2H2O DGo'= -130 kJ/reaction，其它底物也可被转化甲烷，包括甲醇 methanol, 甲酸盐 formate, 甲硫醇 methylcaptan (CH3SH), 乙酸盐 acetate 和甲胺 methylamines。各种厌氧发酵菌协同厌氧分解复杂的有机物为甲烷和 CO2。复杂多聚体通过纤维素分解细菌和其他水解细菌水解单体。初级发酵者发酵葡萄糖，产生各种发酵产物(乙酸盐 acetate, 丙酸盐 propionate, 丁酸盐 butyrate, 琥珀酸 succinate,乙醇 alcohols, hydrogen 和 CO2。H2 可以立即被产甲烷菌、同型乙酸细菌或硫酸盐还原细菌消耗，乙酸可以由一些产甲烷菌转化为甲烷。次级发酵者是使复杂有机物转化为甲烷的主要生物(特别是产氢脂肪酸氧化细菌 the H2-producing fatty acid-oxidizing bacteria)，利用脂肪酸或乙醇为能源。

168. What are primary and secondary fermenters in methane production?

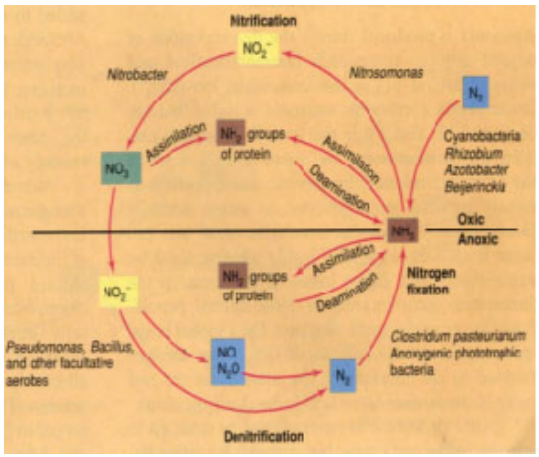
Primary fermenters：consume glucose to a variety of fermentation products (acetate, propionate, butyrate, succinate, alcohols, hydrogen and CO2.

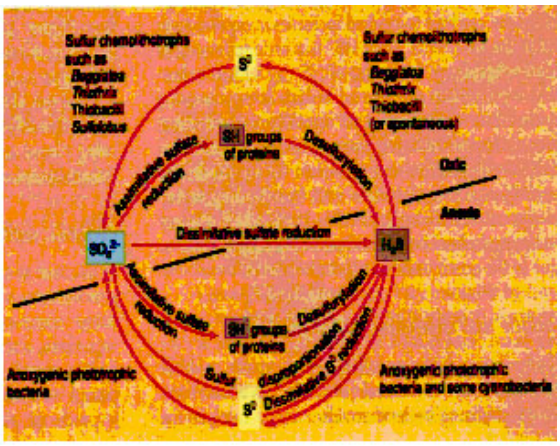
Secondary fermenters：key organisms in the conversion of complex organic materials to methane (espacially the H2-producing fatty acid-oxidizing bacteria).

169.List two syntrophic microorganisms and describe a bit.
两种微生物乙醇发酵菌和产甲烷菌共同作用完成乙醇发酵产生甲烷和乙酸。乙醇发酵菌发酵乙醇产生 H_2 和乙酸（不利于产能反应），产甲烷菌消耗利用乙醇酵解菌产生的 H_2（利于产能反应），总反应为产能反应，产甲烷菌不能直接利用乙醇。

170.How ruminants digest cellulose and starch?
反刍动物具有特殊的器官—瘤胃。在瘤胃里纤维素和淀粉通过特殊的微生物群体的活动而被消化。瘤胃独特特点：体积相当大，处于消化道摄取的食物最先进入的位置，恒定的高温和 PH 值，缺氧环境，或多或少以持续方式作用。纤维素分解菌和原生动物将纤维素水解成纤维二糖和葡萄糖。葡萄糖经微生物发酵产生挥发性脂肪酸（VFSs）。瘤胃细菌和原生动物以及真菌能水解淀粉，发酵葡萄糖，产生有机酸。这些有机酸能通过瘤胃壁进入血液，被生物体氧化作为能源。

171. Schematically describe nitrogen and sulfur cycles and microorganisms involved.





如图 1 所示：全球氮循环中，大气中氮的转移主要通过 N_2 形式，少量以氧化氮 N_2O 和 NO，氨态氮 NH_3。陆地和水体之间氮转移的主要以有机氮，铵离子和硝酸根离子形式。有关微生物：硝化杆菌属，固氮菌属，巴氏梭菌。

如图 2 所示：自然界中有重要意义的只有三种价态的硫，－2（硫氢基，R-SH，和硫化物 SH^-），0（元素硫，S^0）和＋6（硫酸盐，SO_4^{2-}），生物圈中海洋是硫最重要的储藏库（以无机盐形式存在），地球上大量的在沉积物和岩石中是以硫酸盐矿（主要是石膏，$CaSO_4$）和硫化氢矿（主要是黄铁矿，FeS_2）形式存在的。有关微生物：贝氏硫酸菌属，发硫菌属，硫杆菌属。

172. What bacteria is best understood in Bacterial iron reduction and oxidation.
氧化亚铁硫杆菌.(*Thiobacillus ferrooxidans*)

173. Describe the microbial leaching process for recovering copper from CuS.
细菌氧化铜矿可导致铜的溶解，这个过程成为微生物沥滤。沥滤对于 从低等级

矿回收铜是非常重要的。细菌氧化硫化铁矿，即黄铁矿中的铁也是微生物浸矿过程的一个重要部分，以为产生的高铁本身是矿石的一种氧化剂。
Oxidation of copper ores by bacteria can lead to the solubilization of copper, a process called microbial leaching. Leaching is important in the recovery of copper from low grade ores. Bacterial oxidation of iron in the iron sulfide mineral pyrite is also an important part of the microbial leaching process because the ferric iron produced is itself an oxidant of ores.

174. Why some microorganisms are resistant to mercury 水银?
微生物具有几种解除毒素的方法，有一种与 NADPH 相连的酶称为转移 2 个电子倒 Hg^{2+}，使它还原为 Hg^0，这个反应产生的 Hg^0，是挥发性的，但与 Hg^{2+}相比，对人和微生物是无毒的，细菌转化 Hg^{2+}为 Hg^0，然后使更多的 CH_3Hg^+转化为 Hg^{2+}。

175. Microorganisms are sometime used for cleaning of environmental pollutions, such as petroleum and pesticides?
Petroleum can be degraded by hydrocarbon-oxidizing bacteria, because they can oxidize carbon chains to CO_2.
Pesticides may be degraded by genera of both bacteria and fungi, but only their carbon part are degraded to CO_2 finally. Other components are difficult to deal with

176. What is lichen and mycorrhiza?
Lichens are leafy or encrusting growths that are widespread in nature and are often found growing on bare rocks, tree trunks, house roofs, and surfaces of bare soils. It consists a symbiosis of a fungus and an alga.

Mycorrhizae refers to the symbiotic association that exists between plant roots and fungi. It includes ectomycorrhizae and endomycorrhizae.

175.Microorganisms are sometime used for cleaning of environmental pollutions, such as petroleum and pesticides?
Petroleum is a rich source of organic matter and the hydrocarbon within it can are readily attacked aerobically by a variety of microorganism. Bioremediation refers to the clean up of oil or other pollution by microorganism.
hydrocarbon-oxidizing microorganism develp rapidly only in the presence of O_2, as well as other favorable environment condition. Addition of inorganic nutrients can increase bioremediation rates.
Some pesticides can be used as carbon source or electron donor for certain soil microorganism . Significant chlorinated pesticide degradation occur in anoxic environment .the degradation linked to reductive dechlorination of the molecule.
The rate depends on environmental factors ,as well as volatilization, leaching .
Some pesticide are degraded to CO_2, including some completely degradation

occurs only in the presence of other compounds as primary energy source ,a phenomenon called cometabolism or cooxidation .When the degradation is partial ,the product may be more toxic.

176. What is lichen 地衣 and mycorrhizae 菌根?

The lichen plant consist of a symbiosis of two organisms, a fungus and an alga. The alga is phototrophic and is able to produce organic matter used for nutrition of fungus. The fungus provides a firm anchor within which alga can grow. It also facilitates uptake of water and nutrition . lichen is sensitive to pollution.

Mycorrhizae refers to the symbiotic associations that exists between plants roots and fungus. Include ectomycorrhizal in which fungal cell s form an extensive sheath around the root with only little penetration into the root tissue, endomycorrhizae in which fungal mycelium is embedded within the root tissue. Mycorrhizae fungal use simple carbohydrates for growth and have vitamin requirement, obtain carbon from root and get inorganic minerals from soil. It produces plant growth substance.

177. How is Ti-plasmid transfer T-DNA from Agrobacterium to plant genome? (Agobacterium first attached to a wound site, recognized by complementary receptor moleculaes. Then forming large bacterial aggregates on the plant cell.)

The vir genes are the key to T-DNA transfer. The virA gene encodes a protein kinase that interacts with signal molecules and then phosphorylates the product of the virG gene. The latter becomes activated by the phosphorylation event and functions to activate other vir genes. The product of the virD gene has endonuclease activity and nicks DNA in the Ti-plasmid in a region adjacent to the T-DNA. The product of the virE gene is a single strand of T-DNA generated from endonuclease activity and transports this small fragment of DNA into the plant cell. The virB gene product is located in the bacterial membrane and mediates transfer of the single strand of DNA between bacterium and plant.

178. Why is Root nodule bacteria and symbiosis so important for legumes?

Nitrogen fixation by the legume-Rhizobium symbiosis is of considerable agricultural importance, as it leads to very significant increases in combined nitrogen in the soil. Because nitrogen deficiencies often occur in unfertilized bare soils, nodulated legumes are at a selective advantage under such conditions and can grow well in areas where other plants cannot.

Questions for Eukarya

179.List the Eukaryotic Microorganism you have learned.

Eukaryotic microorganism include :

- Algae（藻类）: phototrophic eukaryotic miroorganisms

- Fungi (真菌类) : nonphotosynthetic eukaryotic microorganisms that contain rigid cell walls.
- Slime molds (粘液菌): nonphototrophic eukaryotic microorganisms that lack cell walls and that aggregate to form fruiting structures (cellular slime molds) or mass of protoplasm (acellular slime molds)
- protozoa(原生动物) : unicellular eukaryotic microorganisms that lack cell walls.

180. Describe the differences between various eukaryotic microorganisms.
The differences between various eukaryotic microorganisms are like below:

	藻类 algae	真菌类 fungi	粘液菌 slime molds	原生动物 protozoa
光合作用	Y(oxygenic)	N	N	N
细胞	单细胞或群体	多细胞(除了酵母外)	多/单细胞	单细胞
细胞壁	有（除蜂窝藻外）	有	N	N
运动能力	N	N	某个世代('变形虫'世代)有	Y
叶绿体	Y	N	N	N
子实体	N	Y	Y	N
菌丝体	N	Y	N	N

181. Why do algea have various colors?
In addition to chlorophyll some algae contain other pigment such as carotenoid and xanthophylls
因含有各种不同类型的色素但主要为叶绿体，但当所含的其它色素如胡萝卜素较多时则显红色，或其它颜色。

182. How to classify algae?
- The nature of the chlorophylls present (all algae contain chlorophylla while some of them contain others)
- Carbon reserve polymers produced (starch or several starch derivatives)
- Cell wall structure
- Type of mobility (motility by flagellar movement, no cilia)

183. List six major algae and describe their characteristics you have learned?

Algae group	characteristics			
	Morphology	Pigments	Carbon reserve materials	Cell wall
Chlorophyta (Green algae)	Unicellular to leafy	Chlorophylls a, b	Starch (α-1,4-glucan), sucrose	Cellulose

Euglenophyta (Euglenids)	Unicellular, flagellated	Chlorophylls a, b	Paramylon (β-1,4-glucan)	No wall present
Chrysophyta (Golden-brown algae, diatoms)	Unicellular	Chlorophylls a, c,e	Lipids	Many have 2 over-lapping components made of silica
Phaeophyta (Brown algae)	Filamentous to leafy, occasionally massive and plantlike	Chlorophylls a,c, xanthophylls	Laminarin, (β-1,3-glucan) mannitol	Cellulose
Dinoflagellata (Dinoflagellates)	Unicellular flagellated	Chlorophylls a, c xanthophylls	Starch (α-1,4-glucan)	Cellulose
Rhodophyta (Red algae)	Unicellular, filamentous to leafy	Chlorophylls a, d, phycocyanin, phycoerythrin	Floridean (α-1,4-andα-1,6-glucan) fluoridoside (glycerol-galactoside)	Cellulose

184. What are the differences and similarities between fungal cell wall and bacterial cell wall?

- fungi: resemble plant cell wall architecturally not chemically. Cellulose is present in certain fungi, many have noncellulosic walls. A common constitute is chitin, although other glucan such as mannans, galactosans and chitosans replace chitin in some fungal cell wall,. Generally 80-90% polysaccharide, with protein, lipid, polyphosphates and inorganic ions making up the wall-cementing matrix.
- Gram-negative bacteria: multilayered structure, quite complex, peptidoglycan in periplasm and has outermenbrane.
- Gram-positive bacteria: primarity a single type of molecular peptidoglycan, much thicker. often contain (lipo-) teichoic acid (negative charged).

The differences and similarities between fungal cell wall and bacterial cell wall just like below:

	Fungal cell wall	Bacterial cell wall
differences	含 80-90%多糖(种类繁多) & 多聚磷酸盐	含肽聚糖
	多含几丁质，有些含纤维素	不含几丁质和纤维素
similarities	都含有脂类，蛋白，无机离子	

185. List the types of fungi you have learned.
Three major group:

- Mold，filamentous fungi. 具有菌丝 hypha,可以产生 conidia (无性生殖孢子)和有性生殖孢子。
- Yeasts，单细胞真菌，行分裂生殖。
- Mushroom，大型真菌，产生大型的子实体（fruiting body）和有性生殖孢子 basidiospores

Group: Ascomycetes 子囊菌纲 Basidiomycetes 担子菌纲 Zygomycete 接合菌纲 Oomycetes 卵菌纲 deuteromycetes 半知菌纲
实例：Neurospora, Saccharomyces, Morchella, Amanita, Agaricus, Rhizopus, Allomyces, Penicillium, Aspergillus， Mucor,

186. How to classify fungi?
Classification of fungi based on:

- Morphological properties (形态特征):
  Sexual life cycles(性世代)that the fungi exhibit considerable diversity and the type of sexual spores
- Compositon and characteristic of cell wall

187. What groups do *Saccharomyces, Aspergillus, Mucor, Agaricus* belong to?

Ascomycetes (子囊菌) – *Saccharomyces-yeast*
Basidiomycetes (担子菌) – *Agaricus (伞菌)*
Zygomycetes (接合菌) – *Mucor-molds（毛霉）*
Oomycetes (卵菌) – *Allomyces-mushroom（蘑菇）*
Deuteromycetes (半知菌) – *Aspergillus-molds （曲霉）*

188. Give an example of how cellular slime molds go through their various life cycles.
As cells of dictyostelium become starved, they aggregate and form a pseudoplasmodium, a structure in which cells lose their individuality but do not fuse. The preaggregating vegetative cells which are irregular in shape and lack of orientation become to have regular shape and orientation and start to aggregate, migrate as a cell mass (slug). Fruiting-body formation begins when the slug ceases to migrate and becomes vertically oriented; then it becomes differentiated into a stalk and a head. The cells in the posterior end become spores. On maturation, the spores are released and dispersed on maturation of the head. Each spore gemenates and become a vegetative cell.

189. How do protozoa distinguish themselves from algae, yeast, fungi, and slime molds?

Protozoa is distinguished from

- algae by their greater lack of chlorophyll and cell wall
- yeasts and other fungi by their mobility and lack of cell wall
- slime molds by their lack of fruiting body formation

190. List five groups of protozoa we have learned.

Mastigophora（鞭毛纲）Trypanosoma 锥虫, Sarcodina（肉足纲）amoeba 阿米巴, Ciliophora（纤毛纲）,Balantidium 小袋虫 Euglenoids(眼虫纲 Euglena 眼虫，Apicomplexa（孢子虫纲）Plasmodium 疟原虫

191 Which protozoa causes the African Sleeping Sickness?

Trypanosoma gambiense

It lives and grows in human bloodstream, and can be transmitted from host to host by the tsetse fly, Glossina sp.,（采采蝇，舌蝇） a bloodsucking fly found over in certainparts of Africa. 经过一定的潜伏期，病源体最终会进入大脑和脊髓中枢神经系统，导致炎症致死。

192. What are the unique properties of Paramecium（草履虫）?

(1) Paramecium possesses cilia in some stage of their life cycle which function in motility. Many cilitates have trichocyst which anchored beneth the surface of outer cell layer and enable the cell to attach to a surface and aid in defense by signaling .
(2) Paramecium have two kinds of nuclei:
    a. microneclues :inheritance and sexual reproduction
    b. macroneclues: production of mRNA for various aspects of cell growth and function
(3) Many paramecium species obtain food by ingesting particulate material through a distinct oral region or mouth connected to an underlying gullet.
(4) Many contain endosymbiotic bacteria that in some cases synthesize vitamin or other growth factor.

193. Give an example of one protozoa that causes health problem.

疟原虫（Malaria Parasites）or Plasmodium, which belongs to Apicomplexa（孢子虫纲）, the characteristic of which is lack of motile adult stages.

Food is absorbed in soluble form through outer wall.

Donot form true resting spores, instead producing analogous structures called sporozites

Questions for Archaea

194. List the differences between Bacterial and Archaeal Cell Membrane and Cell Walls.

The differences between bacterial & archaeal cell membrane and cell walls :

	bacteria	archaea

Cell membrane	无聚异戊二烯甘油醚类脂而是磷脂(ester linkage of lipid), 脂肪酸	含聚异戊二烯甘油醚类脂(ether linkage of lipid)
Cell wall	有胞壁酸, D-amino acids	无胞壁酸(muramic acid), 无 D-amino acids only have L-amino acids

Archaea lack fatty acids, instead have hydrocarbon moieties bonded to glycerol by ether (instead of ester) linkages
Glycerol diethers and diglycerol tetraethers are the major classes of lipids present in Archaea
Archaea do not contain muramic acid and D-amino acids, as in Bacteria
A pseudopeptidoglycan is found in some archaea, it consists of two amino sugars: N-acetylglucosamine and N-acetyltalosaminuronic acid, with only L-amino acids linkages
Some contain a thick wall consists only polysaccharide
Some contain cell walls made of glycoprotein
Some lack carbohydrate in their cell walls and have walls consisting of only protein

195. What archaea have you learned so far?

- ✓ Extremely Halophilic Archaeal(极端嗜盐的古细菌)
  Halobacteriales(盐杆菌目)
  -Halobacteriaceae(盐杆菌科)
  -Halobaterium 盐杆菌属
  -Haloferacx 富盐菌属
  -Haloarcula 盐盒菌属
  -Halococcus 盐球菌属
  -Natronobacterium 嗜盐硷杆菌属
  -Natronococcus 嗜盐硷球菌属
- ✓ Methane-producing archaeal(产甲烷的古细菌):
  -Methanobacteriales (甲烷杆菌目)
  -Mehanobacteriaceae(甲烷杆菌科)
  -Methanobacterium 甲烷杆菌属
  -Methanobrevibacter 甲烷短杆菌属
  -Methanothermaceae(甲烷嗜热菌科)
  -Methanothermus 甲烷嗜热菌属
  -Methanococcales(甲烷球菌目)
  -Methanococcaceae(甲烷球菌科)
  -Methanococcus 甲烷球菌属
  -Methanomicrobiales(甲烷微菌目)
  -Methanomicrobiaceae(甲烷微菌科)
  -Methanomicrobium 甲烷微菌属
  -Methanospirillum 甲烷螺菌属
  -Methanogenium 产甲烷菌属

-Methanosarcinaciae 甲烷八叠球菌科
-Methanosarcina 甲烷八叠球菌属
-Methanolobus 甲烷叶菌属
-Methanothrix 甲烷发菌属
-Methanococcoides 拟甲烷球菌

✓ extrememly thermophilic S^0 metabolizers(极端嗜 S^0 代谢菌):

-Thermoproteales 热变形菌目
-Thermoproteaceae 硫还原球菌科
-Thermoproteus 硫还原球菌属
-Thermofilum 热盘菌属

-Desulfurococcaceae 热球菌目
-Desulfurococcus 热球菌科
-theerococcus 热球菌属
-thermodiscus 热盘菌属
-pyrodictium 热网菌属

thermococcales 热热菌目
-thermococcaceae 热球菌科
-thermococcus 热球菌属
-pyrococcus 热球菌属

sulfolobales 硫化叶菌目
Sulfolobaceae 硫化叶菌科
-Sulfolobus 硫化叶菌属-
-Acidianus 酸菌属

✓ CELL-WALL-less archaeobacteria 无细菌壁古细菌
thermoplasma 热原体属

✓ archaeobacterial sulfate reducers 降解硫酸盐的古生细菌

Archaeoglobale 古生球菌目
Archaeoglobaceae 古生球菌科
-Archaegolobus 古生球菌属

196. Where to isolate halophilic Archaea?

高盐浓度的自然界，如盐湖，硷湖和盐场，也常见于晒制的粗盐和用晒盐腌制的蛋白质品(咸鱼和皮革)。

197.What are the physiological features of halophiles?

All are chemoorganotrophs.
Most are obligate aerobes
All require large amount of sodium for growth
All strain gram negatively,binary fission growth
Most are nonmotile
Halobacterium and halococcus contain large plasmid
Peptidoglycan replaced by plycoprotein in the cell wall
Cellular components exposed to the external environment require high Na+ for

stability
Cellular internal component require high k+ for stability
Na+ stabilize the cell wall

The physiological features of halophiles are:
细胞杆状或球状，并出现许多退化类型，从圆盘形到三角形都有。不运动或靠丛生极端鞭毛运动。革兰氏染色阴性和阳性(2%乙酸固定后) 。好氧或兼性厌氧。需要很高浓度的盐，生长至少需要 1.5mol/L(约为 9%) NaCl, 但大多数菌种在 3.5-4.0mol/L NaCl 生长最好。由于细胞内含有 C_{50} 类胡萝卜素(菌红素) ，使其菌种落具有各种红色色调，并使得该菌在自然环境中群体生长时现红颜色。视黄醛类色素在这类细菌中可能是普遍存在的，它们能使离子运动并穿过细胞膜。含菌视紫素者,可用菌视紫素作为质子 pump，被光能所驱动所产生的质子梯度合成 ATP。最适的生长温度是 35-50°C。化能有机营养，利用氨基酸或碳水化合物作为碳源。在细胞内，通过积累高浓度的 KCl 来实现其渗透调节。

注：关于 rhodopsin
视紫素在嗜盐菌中发挥重要功能。共有四种 rhodopsin 起作用：

- bacteriorhodopsin, 用于接受光照，向胞外泵出 H 离子，产生质子浓度梯度，并借助 proton motive force 合成 ATP；
- halorhodopsin, 光驱动的排钾吸氯质子泵，保证体内的 NaCl 水平
- 两种 sensory rhodopsin, 感光作用，控制向光性。

198. What are the three major classes of methane-producing archaea?
The three major classes of methane-producing archaea can be sorted by the substrates.

- CO2-type substrate consumers
  $CO_2+H_2 \rightarrow CH_4+H_2O$
- reduction of methyl group of methyl-containing compounds to methane
  $CH_3OH+H_2 \rightarrow CH_4+H_2O$
  In the absence of H_2 $CH_3OH \rightarrow CH_4+CO_2+H_2O$
- grow on acetate rather than methanol for making methane.
  $CH_3COO^-+H_2O \rightarrow CH_4+HCO_3^-$

可以通过三种基质产生甲烷：

- 二氧化碳和氢气
- 含甲基的化合物，如甲醇等
- 有机酸类，如醋酸等

199 List several species of methane-producing archaea you have learned.
Methanobacterium formicicum 甲酸甲烷杆菌,
Methanobrevibacter ruminantium 反刍甲烷短杆菌，
Methanothermus fervilus 沸腾甲烷嗜热菌,
Methanococcus vanniellii 万尼氏甲烷球菌,
Methanomicrobium mobile 运动甲烷微菌,

Methanospirillum hungatei 洪氏甲烷螺菌,
Methanogenium cariaci 卡利亚奇产甲烷菌,
Methanosarcina barkeri 巴氏甲烷八叠球菌,
Methanolobus tindarius 丁氏甲烷叶菌,
Methanothrix soehngenii 索氏甲烷发菌,
Methanococcoides methylutens 甲基甲烷类球菌

****200 List several unique methanogenetic coenzymes we have learned.
辅酶 M(Coenzyme M)
辅酶 F420(Coenzyme F420)
辅酶 F430(Coenzyme F430)
亚甲基喋呤(mehanofuran),也就是 MF, methanopterin,
7-mercaptoheptanoylthreonine phosphate (HS-HTP)

201 Describe the general properties of hyperthermophilic archaea you have learned.
细胞杆状，丝状或球状。大多数严格厌氧。专性嗜热，最适生长温度为 70-105°C。嗜酸性和嗜中性。化能有机营养或化能无机营养代谢。大多数种代谢硫。

202 Describe at least four hyperthermophilic archaea you have known.
Thermofilum pendens 依赖热丝菌-
细胞呈细杆状，长度变化大。常在细胞两端形成球状突起物，有时还形成膨大的部分，空细胞可变成螺旋状。革兰氏染色阴性，厌氧，专性化能无机营养，无机营养，嗜热嗜酸。通过硫的呼吸作用多肽。最适生长温度为 85-90°C，最适 pH 值约为 5。
Desulfurococcus mucusus 粘质脱硫球状菌-
细胞呈球状，细胞壁柔韧，革兰氏染色阴性。通过硫的呼吸作用或发酵，可利用蛋白质，多肽或碳水化合物。温度 97°C 和 pH2.2-6.5。
Sulfolobus acidocaldarius 嗜酸热硫化叶菌-
细胞呈球状，形状高度不规则，形成独特的裂片，通常单个细胞存在。细胞可紧紧地粘附在硫结晶体上，细胞壁由以六边形排列的蛋白质亚单位组成。好氧，可氧化硫，硫化物或连四硫酸盐形成硫酸，并固定 CO2 作为碳源，进行无机营养生长。也能氧化复杂的有机物质，糖或氨基酸，进行有机营养生长。有些菌株在有氧条件下能氧化 Fe2+成 Fe3+。嗜热嗜酸，能在 50-87°C 和 pH1-6 的条件下生长。
Acidianus infernus 下层酸菌-
细胞呈球状，形状高度不规则，几乎只以单个细胞存在。细胞壁由以六边形排列的蛋白质亚单位组成。兼性厌氧。在好氧条件下，通过元素硫的氧化作用，或者在厌氧条件下，通过元素硫和 H2 的还原作用，进行无机营养生长。嗜热嗜酸，能在 65-95°C 和 pH1-6 的条件下生长。耐盐，最适生长的 NaCl 浓度为 0.2%左右，最高浓度为 4%。

203 There are two bacteria that exhibit hyperthermophilic tendencies. Describe them a bit.
Aquifex 产液菌属- 专性化能无机营养的超嗜热细菌，在微好氧条件下氧化氢或还原态硫化物。最适温度约为 85°C，而且能在 95°C 生长。
Thermotoga 栖热袍菌属- 严格厌氧的能发酵的细菌，生长在 55-90°C，最适温度约为 80°C。该菌产生独特的脂类，内含极其长链的脂肪酸，但其细胞壁含有肽聚糖这一细菌的标记分子。

204 What is Thermoplasma?

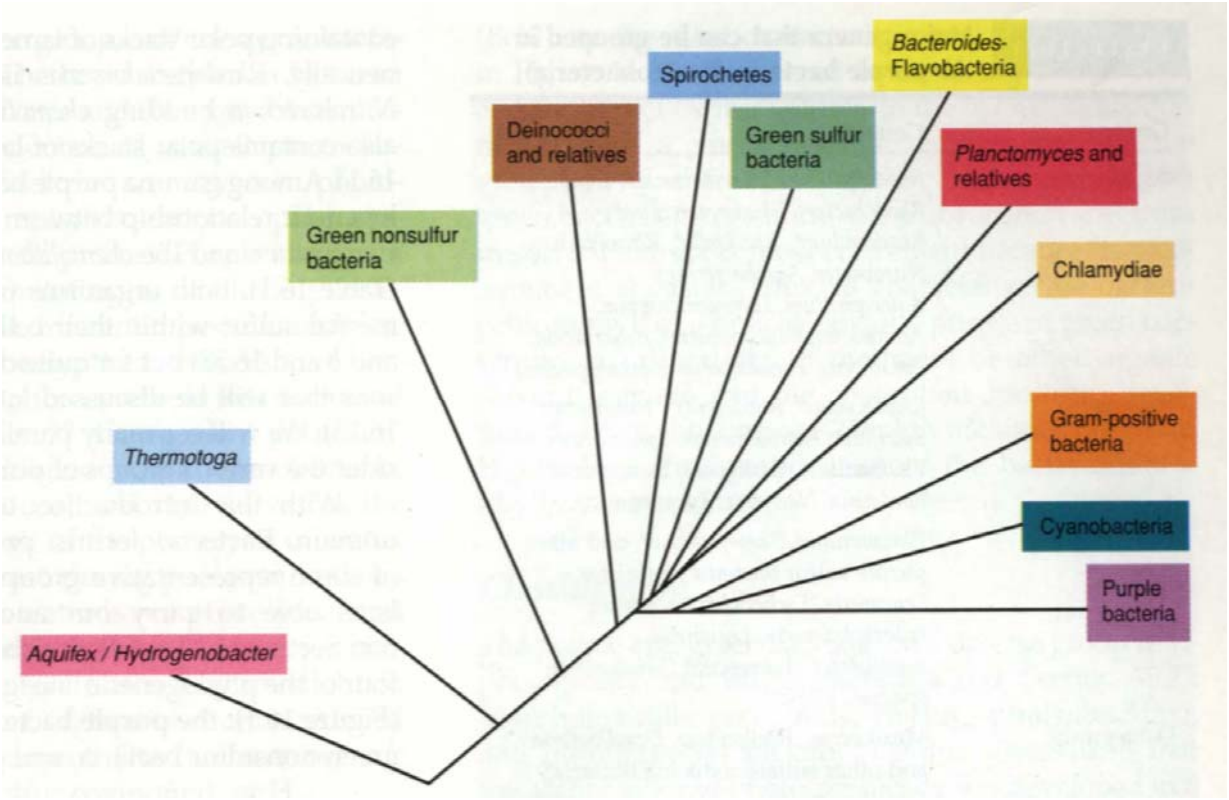
Thermoplasma(热原体属) ：

1) 无真正细胞壁，仅由 1 个三层膜所包围，约 5-10nm 厚。
2) 细胞有多种形状。
3) 细胞膜为了能在无细胞壁条件下抵御渗透压和低 pH 和高温双重极端环境,进化出特殊化学结构。含有脂多糖，这种脂多糖是一种带有甘露糖和葡萄糖单位的四醚类脂。质膜也含有糖蛋白，但没有固醇类物质。
4) Gram-negative
5) 嗜酸，好氧，化能有机营养和嗜热。
6) 基本上（一种例外）适宜在 55°C，pH2 的合成培养基上生长。
7) 基因组很小，GC 含量约 46%，被高度碱性的 DNA 结合蛋白包围构成似真核细胞核小体的球状颗粒。其碱性的 DNA 结合蛋白与 Histone 的 A.A Sequence 同源性高。

205. What seems to be the maximal temperature for life to exist?
140-150 °C

206. Why it is said that archaea may be the earliest life on earth?
The early geochemical conditions in earth are: high temperature, high salt, low pH, strict anoxic condition. Most of the archaea can live in the high temperature (e.g. thermopasma) else high salt (e.g. halophiles) else low pH(e.g. acidianus) and normally are anoxic microorganism.

207 Draw the phylogenetic tree of the major lineages of Bacteria based on 16S ribosomal RNA Sequence comparisons.



208. How many groups of purple bacteria you have learned?
可以行驶 anoxygenic photosynthsis, 无氧条件下进行光合作用。因为在有氧条件下，氧气作为电子受体结合电子形成水，二氧化碳结合电子的能力弱于氧气。在无氧条件下以二氧化碳作为电子受体进行光合作用。
分为 purple sullfur bacteria 和 purple nonsulfur bacteria 两类。
前者消耗负二价硫离子作为电子供体，后者利用有机质作为碳源和电子供体。多数的 PNSB 也可固定氮气作为氮源。

209 Describe the differences between purple bacteria, green bacteria an cyanobacteria?
Purple bacteria:
Bacteriochlorophylls a, b
Anoxygenic photosynthesis
One photosystem

Green bacteria
Bacteriochlorophylls c, d or e
Anoxygenic photosynthesis
One photosystem

Cyanobacteria
Bacteriochlorophylls a

Oxygenic photosynthesis （有氧光合作用）
Two photosystems

210 Why do purple bacteria, green bacteria and cyanobacteria have various colours?
That is because they have different bacteriochlophylls which differs in substituents on various parts of the porphyrin ring.

211. Describe the differences between nonsulfur purple bacteria and sulfur purple bacteria.
Nonsulfur purple bacteria:

- Can only use sulfide in a low concentration;
- Have great photoheterotrophic abilities;
- Some have ability to utilize methanol as sole carbon source for phototrophic growth
- Most are active N_2 fixer

Sulfur purple bacteria

- Commonly found in anoxic zone of lakes as well as in sulfur spring
- Deposit sulfur, and oxidize it to sulfate
- Limited ability to use organic compounds as carbon source for phototrophic growth
- Some species grows chemoorganotrophically on acetate.
- can live in high concentration of sulfide

212. List four purple sulfur and nonsulfur bacteria you know, respectively.
Purple non-sulfur bacteria

- Rhodospirillum红螺菌属
- Rhodopseudomonas 红假单胞菌属
- Rhodobacter红细菌属
- Rhodovulum小红卵菌属

Purple sulfur bacteria

- Ectothiorhodospira 外硫红螺菌属
- Chromatium 红硫细菌属
- Thiocapsa荚硫细菌属
- Thiocystis 囊硫细菌属

213. Describe the differences between green nonsulfur bacteria and green sulfur bacteria.
Green sulfur bacteria

- Morphologically diverse

- Strictly anaerobic
- Obligate phototrophic
- Most can assimilate simple organic substances (aetate, propionate, pypuvate and lactate) for phototrophic growth, provided that sulfur compound is present as a sulfur source.

Green nonsulfur bacteria
- Chloroflexus has been given the desination green nonsulfur bacterium
- can grow chemoorganotrophically in dark under aerobic conditions
- can grow phototrophically on a wide variety of sugars, amino acids, and organic acids
- can grow phototrophically with H2S, H2 or CO2
- best grow photoheterotrophically

214. List three green sulfur and green nonsulfur bacteria you know, respectively.
Green sulfur bacteria:
- Chlorobium limicola, 泥生绿菌
- Pelodictyon clathratiforme 格形暗网菌
- Prosthecochloris突柄绿菌属
- Ancalochloris 绿臂菌属

Green nonsulfur bacteria
- Chloroflexus aurantiacus 橙色绿屈挠菌
- Oscillochloris 绿颤蓝细菌属
- Heliothrix 螺丝菌属

215. What are the uniqueness in heliobacteria?
They are strictly anaerobic phototrophs, which cantain bacteriochlorophyll g instead of bacteriochlorophyll a, unable to grow by respiratory means and can grow in darkness by pyruvate fermentation

216 How are cyanobacteria grouped?
Bergey's manual has divided them into 5 major groups depending on its morphological diversity.
- Unicellular,divideing by binary fission
- Pleurocapsolean: reproduce by formation of spherical cells called baeocytes produced through multiple fission.
- Oscillatorian filamentous cell that divide by binary fission in a single plane
- Nostocalean filamentous cell that produce heterocytes
- Braceing cell divide to form branches

217. What is heterocyst in cyanobacteria?
They are sole sites of nitrogen fixation, which are rounded, distributed

regularly along a filament or at one end of filament (it is the sole site of nitrogen fixation in heterocystous cyanobacteria, which lacks photosystem II).

218. What is prochlorophyte?
They are phototrophic prokaryotes, which contain chlorophyll a and b, but no phycobilins (藻胆素，可溶于水的蛋白质类色素). They resemble both cyanobacteria (because they are prokaryotic and produce chlorophyll a) and green plant/alga chloroplast (because they contain chlorophyll b instead of phycobilins).

219.List three nitrosifying and nitrifying bacteria, respectively.
Nitrosifying bacteria:亚硝化细菌(把氨转化为硝酸)
- Nitrosomonas,亚硝化单胞菌属
- Nitrosococcus 亚硝化球菌属
- Nitrosospira 亚硝化螺菌属

Nitrifying bacteria:（硝化细菌，把亚硝酸转化为硝酸）
- Nitrobacter 硝化杆菌属
- Nitrospina 硝化刺菌属
- Nitrococcus 硝化球菌

220. Give two species of sulfur and iron oxidizing bacteria and tell what they can do.
Thiobacillus : leaching of the release of Fe from sulfur mineral and other heavy metals associated with the pyrite（黄铁矿）.
Beggiatoa:can cause major setting problem insewage treatment facilities and inindustrial waste lagoons

223. List three sulfate- and sulfur-reducing bacteria you have learned, respectively.
Sulfate-reducing bacteria can be divided into two large physiological subgroups:
a. utilizing lactate, pyruvate, ethanol, or certain fatty acid as carbon and energy source to reduce sulfate to hydrogen sulfide, such as *Desulfovibrio*.
b. specialized in the oxidation of fatty acids, particularly acetate, reducing sulfate to sulfide, such as *Desulfobacter.*
Sulfur-reducing bacteria: Such as Desulfuromonas

224. Describe two homoacetogenic(同型产乙酸) bacteria you have learned and what are their physiological properties?
Acetobacterium woodii ; *Clostridium aceticum*;
Physiological properties
- Obligate anaerobes;

- Gram-positive;
- CO_2 as a terminal electron acceptor
- Acetate as the sole product of anaerobic respiration
- Acetyl-CoA pathway convert CO_2 to acetate

225. What are prosthecate bacteria? Are flagellates prosthecae? Why?
Prosthecate bacteria are appendages cell that form prothecae （前鞘） used for attachment or nutrient absobtion. They are primarily aquatic.
Flagellates are not prosthecae, because prosthecae contain cytoplasm and cell wall and its function is for attachment and nutrient absorption. F lagellates are protein complex inserting into the cytoplasm menbrane whose function is for motibility.

226. List two spirilla you have learned, give their Chinese names too.
Spirillum; 螺旋菌属
Bdellovibrio; 蛭弧菌属
Campylobacter; 弯曲杆菌属
Azospirillum 固氮螺菌

227. Bdellovibrio is a group of interesting bacteria, what are their unique properties?
Preying on other bacteria, using as nutrients the cytoplasmic constituents of their gist,
Unique mode of attack and develop intraperiplasmically
A wide variety of gram-negative bacteria can be attacked by a single Bdellovibrio species. Gram-positive cells are not attacked.
It doesn't assimilate nucleotides, fatty acid and even whole protein. It only grows on living prey except some mutant species.
注：
bdellovibrio 在其他细菌中寄生，并将诉诸细菌消化。黏附到其 prey cells 后，Bdellovibrio 穿透宿主细胞的细胞壁，并在 periplasm 中复制形成 bdelloplast。许多的 Gram- 细菌可被 bdellovirio 寄生，而 Gram+ 则不会被寄生。

228. Give the names of two important spirochetes and illustrate the importance to study them.
Treponema, (Commensal or parasitic in humans, other animals, can cause Syphilis, yaws, swine dysentery, pinta)
Borrelia, (lives in humans and other mammals, arthropods, and can cause Relapsing fever, Lyme disease, ovine and bovine borreliosis)
It'll be of great help to learn them well, in order to find good cure for this disease.
Spirochetes are widespread in aquatic environments and in and some of them cause diseases including syphilis.

229. Why is gliding myxobacteria interesting?
They lack flagella but are able to move when in contact with surfaces. Some of them posses the interesting property of forming multicellular structure called fruiting body and show complex developmental life cycle involbing intercellular communication. Myxobacteria are chemoorganotrophic soilbacteria that by consuming dead organic matter or other bacterial cells.

230. What is the sheathed bacteria?
They are filamentous bacteria with a unique life cycle involving formation of flagellated swarmer cells within a long tube or sheath.

231. Tell as much as you know about Pseudomonads.
Straight or curved rods but not vibroid;size 0.5-1.0um by 1.5-4.0um;no spores
Purple bacteria(proteobacteria), gram-negative
Polar flagellated, aerobic, grow at neutral pH mesophilically
Some are Chemoorganotrophic, using h2 or CO as sole electron donor.never show a fermentative metabolism(although may produce small amount of acid from glucose aerobically)
P. aeruginosa is opportunistic pathogen, some are plant pathogens.
No spores, motile always, no sheaths, appendages, or buds
Use low molecular-weight-organic compounds, not polymers
Some use nitrate as electron acceptor anaerobically; some use arginine as energy source anaerobically

232. List at least three major genera of free-living, aerobic and nitrogen-fixing bacteria.
- Azotobacter
- Azomonas
- Azospirillus
- Beijierinckia
- Derxia

233. How can acetic acid bacteria be used for practical purposes?
Used in the commercial production of vinegar
Exploited in the manufacture of ascorbic acid (vitamin c)
Used in the production of pure cellulose

234. Why is Zymomonas interesting?
Zymomonas carries out fermentation of sugar to ethanol, used in beverage industry because it shows higher rate of glucose uptake and ethanol production than many yeast.
What's more, it can tolerate high concentration of ethanol. As it is gram negative, zymomonas can be operated conveniently in gene engineer, which draws people strong interests.

Zymomonas 为发酵工业常用菌种，用于将葡萄糖酵解为酒精。但其代谢途径为 Entner-Doudoroff pathway.

235. What are the major genera of vibrio and related genera? Why do some fishes emit light?

- Vibrio
- Photobacterium
- Aeromonas
- plesiomonas

They are gram negative, facultatively aerobic rods and curved rods that possess a fermentative metabolism.
These fishes posssess a special organ in which bioluminescent bacteria grow. So we see that they emit light.

236. List as many as possible the enteric bacteria you have known. What diseases they may cause?

- Escherichia: diarrhea, children's nurseries, urinary infections, enterotoxin.
- Salmonella: typhoid fever, gastroenteritis.
- Shigella: gastroenteritis, endotoxin, neurotoxin.
- Proteus: urinary tract infections, enteritis, kidney infection.
- Yersinia: bubonic plague, tuberculosis-like disease in animals, intestinal infection.
- Enterobacter;edwardsiella;kelbsiella;Arizona;citrobater;providencia;hafnia

Disease

- diarrhea 腹泻、痢疾;
- urinary infection 尿路感染
- gastroenteritis 肠胃炎
- bacillary dysentery 杆菌性痢疾
- typhoid fever 伤寒
- enteritis 肠炎
- kidney infection 肾脏感染

237. What Neisseria species causes sexual disease?
Neisseria gonorrhoeae(淋病奈瑟球菌)

238. Compare the differences and similarity among Rickettsia, Chlamydia and Viruses
Answer:

	Rickettsia	Chlamydia	Viruses
Nucleic acid	RNA and DNA	RNA and DNA	RNA or DNA never both
Ribosome	Present	present	Absent

Cell wall	Peptidoglycan present, containing DPA	Peptidoglycan present, but no DPA	No wall
Structure integrity during replication	Maintained	maintained	Lost
Synthesizing large molecules	Carried out	Carried out	With use of host machinery
ATP synthesis system	Present	Some have	Absent
Oxidize Glu	Yes	no	No
Sensitivity to biotics	Sensitive	Sensitive(except for penicillin)	Resistant
Capable of oxidizing glutamate	Y	Y	N
Synthesis	Carried out	Carried out	Host machinery

Similarity: They are all obligate intracellular parsites. 严格的胞内侵染

239. What are the major groups of Gram-positive bacteria?

- Nonsporulating, low GC,革氏阳性
- Endospore-forming, low GC，革氏阳性
- Cell wall-less, low GC 革氏阳性
- High GC 革氏阳性
- Filamentous, High G，革氏阳性

240. List two gram-positive Cocci you have learned.

Staphylococcus 葡萄球菌
Micrococcus 微球菌
Sarcina 八叠球菌

241. What is the uniqueness of *Deinococcus radiodurans*?

Many are more resistant to radiation than bacteria endospore, alse resistant to mutagenic chemicals.
Treatment of a sample with strong does of radiation effectively sterilizes the sample of organism other than *Deinococcus radiodurans making isolation of deinococcus relatively strainghtforward*
对于 UV 辐射和化学试剂诱变有较强的抗性

242. List at least three lactic acid bacteria you have learned, both in English and Chinese.

- Streptococcus(链球菌属)
  Leuconostoc
  Pediococcus
- Lactobacillus（乳杆菌属）

Enterococcus
- Lactococcus（乳球菌属）

243. What group of bacteria are gram-positive, endospore-forming bacteria?
- Bacillus
- Clostridium

257. Which bacteria can be used as bioinsecticides （生物杀虫剂）?
- Bacillus *popilliae*
- Bacillus *thuringiensis*

258. Differences between protoplasts and Mycoplasma?
Mycoplasmas resembles protoplast in their lack of cell wall,but they are more resistant ot osmotic lysis and are able to survive conditions under which protoplast lyse. This ability to resist osmotic lysis is at least partially determined by nature of mycoplasma cytoplasmic menbrane, which is more stable than other prokaryote.

259. What are the major groups of the High GC Gram-Positive Bacteria: "Actinomycetes" 放线菌?
- Coryneform group of bacteria
- Propionic acid bacteria
- Obligate anaerobes
- Actinomycetes

260. List two major groups of corynebacterium 棒状杆菌.
- Corynebacterium
- Arthrobacter

261. Where was propionibacterium fast discovered?
Swiss cheese

262. What is the bacteria that causes tuberculosis 肺结核?
Mycobacter tuberculosis

263. What is streptomycete 链霉菌 so interesting?
The streptomycete spores are produced by formation of cross-wall in the multinucleate sporophores 多核的芽孢柄(子实体)内 followed by seperation of the individual cells directly into spores.
They are primary soil microorganisms, produce earthy odor 泥土的气味, and are the most important antibiotic producers.