武漢大學 WUHAN UNIVERSITY

分子生物学

共三位老师上课：肖锐，殷浩，张竹珍

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For WHU Undergraduates (48 class hours)
Spring Semester, 2022

注：本节课件以陈亮老师PPT为基础，进行了部分调整

教师简介：张竹珍

教育经历：
2002-2006 长安大学应用化学系 学士
2006-2012 中科院上海生科院 博士
2012-2015中科院上海生科院 博士后
2015-2017美国西南医学中心，博士后
工作经历：
2017-2021美国西南医学中心，助理讲师、讲师
2022-至今 武汉大学生命科学学院 教授

目前主要研究领域和兴趣：
皮肤细胞的代谢与皮肤发育及皮肤相关疾病

Molecular Biology in Wuhan University

- **Obligatory course for biology students**
- **Intended for sophomores and juniors**
- **Prerequisite: General biology, Biochemistry**

参考书籍

Watson et al,. Molecular Biology of the Gene, (Sixth Edition)

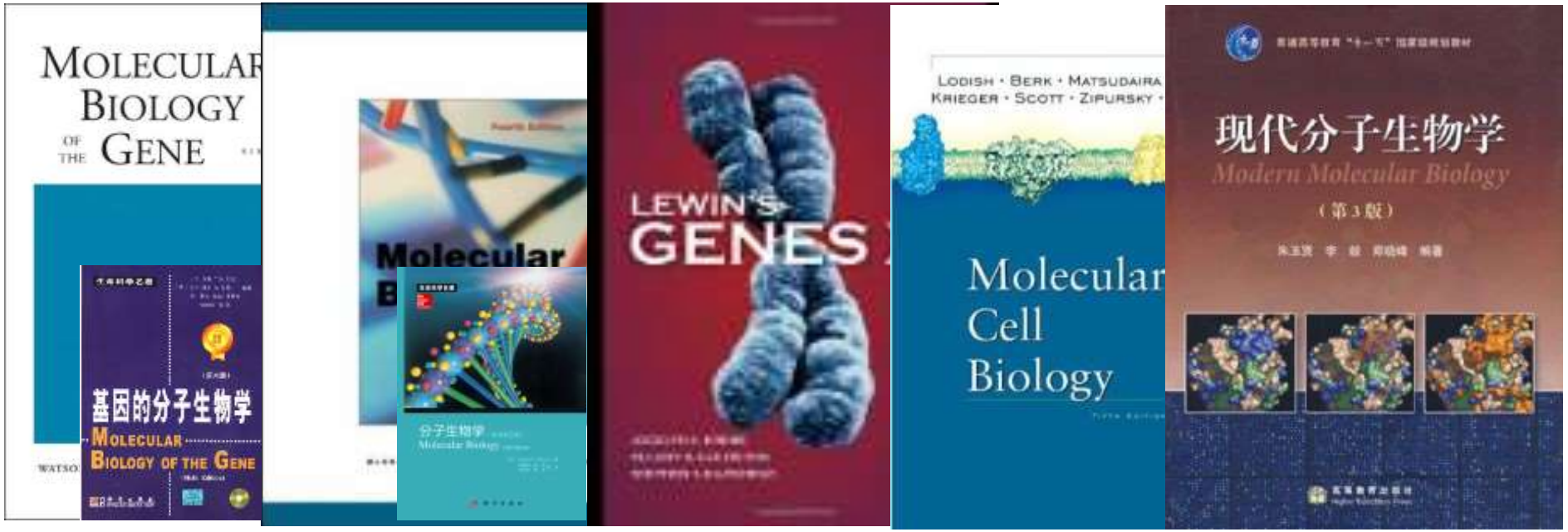
Robert F. Weaver Molecular Biology, (Fifth Edition)

分子生物学（第五版）（郑用琏等译）科学出版社

Lewin's Genes XII

Lodish et al,. Molecular Cell Biology, (Fifth Edition)

现代分子生物学（第五版）（作者:朱玉贤 李毅 郑晓峰）高等教育出版社



考试规则

考试的目的是督促学习与检查教学效果,但考场的表现反映一个人的基本素质和诚信意识

（1）不离开自己的座位。（2）合上书本和笔记。（3）个人只能看和写自己的试卷,不得左顾右看或交头接耳（4）老师宣布停的时候，所有学生必须放下自己的笔，目视前方。（5）老师宣布收试卷时，试卷必须快速由每排的一端传到另一端。

课堂小考: 如果违反以上任何一条规则，第一次违规者，本次考试成绩为零，第二次违规者必须重修该课。缺席不补考,本次成绩为零，但如果提前请假可以得当次考试的最低分。

期末考试：任何作弊者，一经发现，立刻取消考试资格，重修此课。

Evaluation system:

1. In class quizes - 20% (20 points in 100 system, 3-4 times)
2. Topic presentation (ppt format, 10 groups, point to each other) – 10%
3. Final exam: - 70%
4. Bonus points: 1-5 points

Ahaa, your final results might exceed 100 points!!!

Rules in class :

1. 上课不能交头接耳
2. 手机等关机或震动、静音（不能接）
3. 提问先举手
4. 迟到5分钟，请不要进来
5. 欢迎提问，但不要让老师帮你做作业

课程安排：

	日期	时间（周五）	学时	授课内容	授课老师	
第一节	2月24日	18:30-20:55	3	Introduction		
第二节	3月3日	18:30-20:55	3	Chapter 6-7 – The structure of DNA, RNA and genome		
第三节	3月10日	18:30-20:55	3	Chapter 8-The replication of DNA		
第四节	3月17日	18:30-20:55	3	Chapter 12-Mechanism of transcription in bacteria		
第五节	3月24日	18:30-20:55	3	Chapter 12-Mechanism of transcription in eukaryotes		
第六节	3月31日	18:30-20:55	3	Chapter 13-RNA splicing		
第七节	4月7日	18:30-20:55	3	Chapter 14-15 Translation and the genetic code		
第八节	4月14日	18:30-20:55	3	Chapter 16-Transcriptional regulation in prokaryotes		
第九节	4月21日	18:30-20:55	3	Chapter 17-Transcriptional regulation in eukaryotes		
第十节	4月28日	18:30-20:55	3	Chapter 20-Techniques of Molecular Biology-Part I-核酸相关技术与工具酶		
第十一节	5月5日	18:30-20:55	3	Chapter 20-Techniques of Molecular Biology-Part II-载体，蛋白相关技术与蛋白-核酸相互作用研究		
第十二节	5月12日	18:30-20:55	3	Chapter 20-Techniques of Molecular Biology-Part III-Gene editing and qPCR		
第十三节	5月19日	18:30-20:55	3	Chapter 18-Regulatory RNA and Chapter 21-Model organism		
第十四节	5月26日	18:30-20:55	3	Presentation and Discussion-Part I		
第十五节	6月2日	18:30-20:55	3	Presentation and Discussion-Part II		

张竹珍 1-4节
肖锐　5-9节
殷昊　10-13节

第一节：分子生物学绪论

本节教学内容：

1、分子生物学概念
2、分子生物学研究内容
3、分子生物学发展历程
4、分子生物学未来发展趋势

教学目的：

1、了解什么是分子生物学
2、掌握分子生物学的基础知识
3、了解分子生物学发展的历程，以及重要的发现及其意义
4、熟悉当今分子生物学的应用，以及未来发展趋势

分子生物学的概念

What is Molecular Biology?

Molecular biology seeks to explain the relationships between the structure and function of biological molecules and how these relationships contribute to the operation and control of biochemical processes.
---Turner et al.

Molecular biology is the study of genes and their activities at the molecular level, including transcription, translation, DNA replication, recombination and translocation.
--- Robert Weaver

分子生物学是研究核酸、蛋白质等生物大分子的形态、结构特征及其重要性、规律性和相互关系的科学，是人类从分子水平上真正揭示生物世界的奥秘，由被动地适应自然界转向主动地改造和重组自然界的基础学科。现代分子生物学 --- 朱玉贤、李毅

分子生物学研究内容：

主要研究核酸、蛋白质等所有生物大分子的形态、结构特征及其重要性、规律性和相互关系的科学

现带分子生物学囊括：

重组DNA技术（基因工程）

基因表达调控研究（包括CRISPR基因编辑）

生物大分子的结构功能研究（结构生物学）

基因组、功能基因组与生物信息学研究

相互穿插联系，密不可分

分子生物学与生物化学、细胞生物学区别

生物化学： 侧重生命的化学本质研究，如生物体内化学组成、结构、作用的化学本质和机理，及生命过程各种化学反应过程、调控及功能测定等

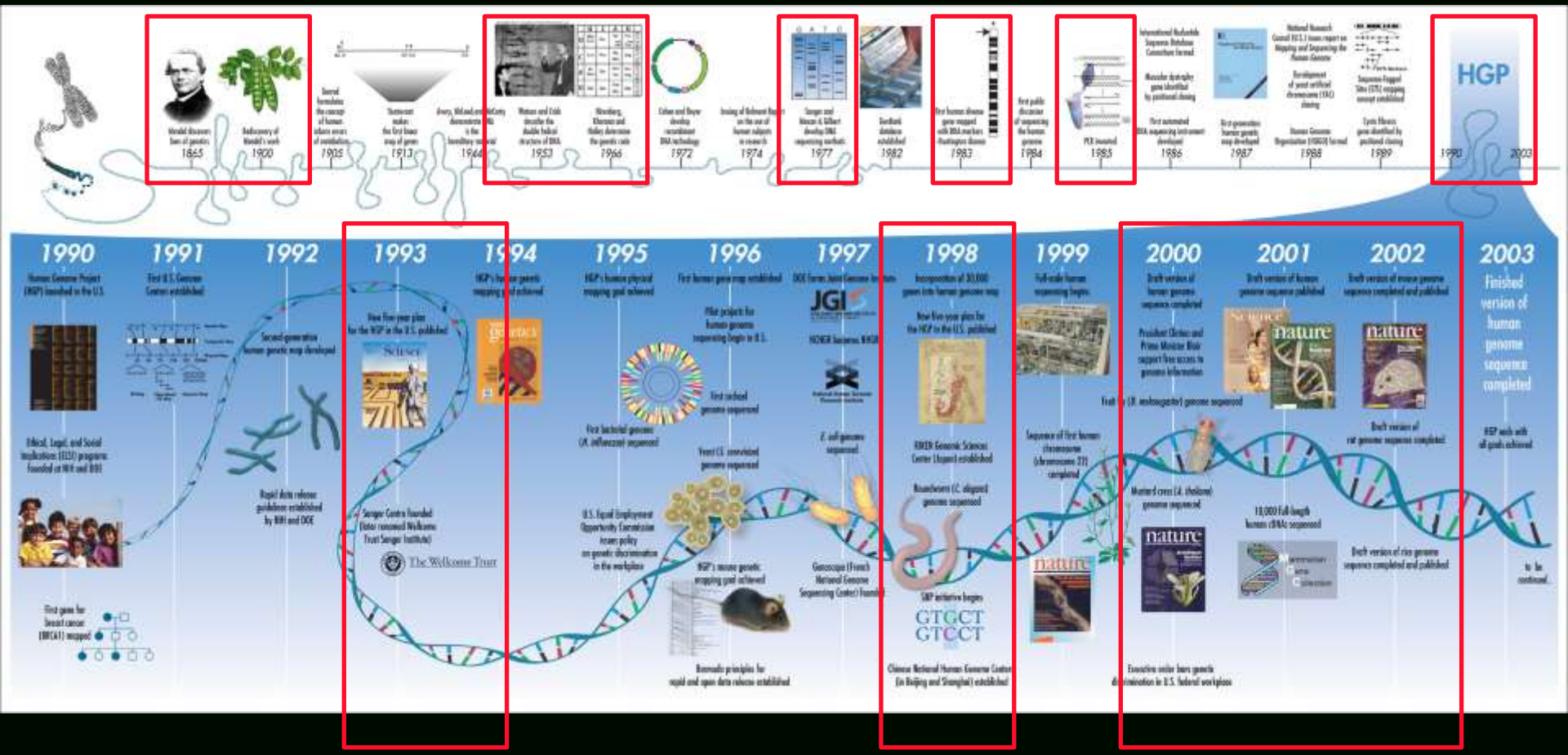
分子生物学： 侧重在分子水平上研究生物大分子(如核酸、蛋白质)的结构、功能、生物合成等，从而阐明生命现象的本质。可以归为生物化学的一个分支。

细胞生物学： 侧重以细胞为研究对象，从整体、显微、亚显微、分子水平研究细胞、细胞器的结构和功能，分子的细胞内定位、细胞信号转导等

分子生物学发展相关重要历史事件

- 1859年——达尔文（英国）——发表《物种起源》——确立了进化论的概念。
- 17世纪末——安东尼·列文虎克（荷兰）——制作第一架光学显微镜。
- 17世纪末——罗伯特·胡克（英国）——引入“细胞”这一概念。
- 十九世纪末——施莱登（德国植物学家）施旺（德国动物学家）——推动细胞学说。
- 十九世纪末——孟德尔（奥地利）——提出遗传学定律。
- 十九世纪末——米歇尔（瑞士）——发现核素（脱氧核糖核苷酸DNA）
- 二十世纪初——摩尔根（美国）——实验证明了“基因学说”
- 1928年——格里菲斯（英国）——肺炎双球菌转化实验。埃弗里（美国）——实验证明基因是DNA分子。
- 1936年——萨姆纳（美国）——证明酶是蛋白质。
- 1952年——赫希（美国）蔡司（美国）——噬菌体侵染实验。
- 1953年——沃森（美国）克里克（英国）威尔金斯（英国）富兰克林（英国）——发现DNA双螺旋结构。
- 1953年——桑格（英国）——纸层析技术阐明胰岛素一级结构，开创了蛋白质序列分析的先河。
- 1954年——克里克（英国）——提出中心法则。
- 1958年——梅塞尔森（美国）斯塔尔（美国）——证明了DNA半保留复制。
- 1968年——尼伦伯格（美国）——破译遗传密码。
- 1980年——桑格（英国）——设计一代DNA测序法（双脱氧核苷酸测序）。
- 1983年——麦克林托克（美国）——发现转座基因（jumping gene）。
- 1986年--------PCR技术
- 1989年——奥特曼（美国）切赫（美国）——发现核酶。
- 1997年——布鲁希纳（美国）——发现朊病毒。
- 2000年——布莱克本（澳大利亚）——揭示端粒和端粒酶机制
- 2001年-------人类基因组测序初步完成
- 2012年--------CRISPR系统被用于基因编辑

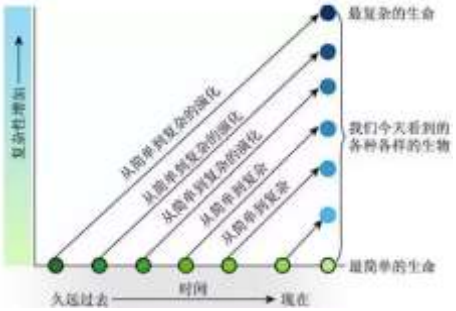
An Odyssey to Understand Life



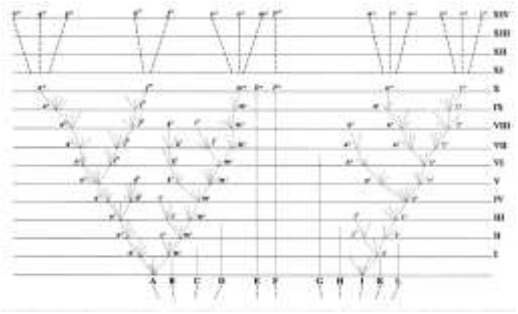
What is the basis for life?

神创论

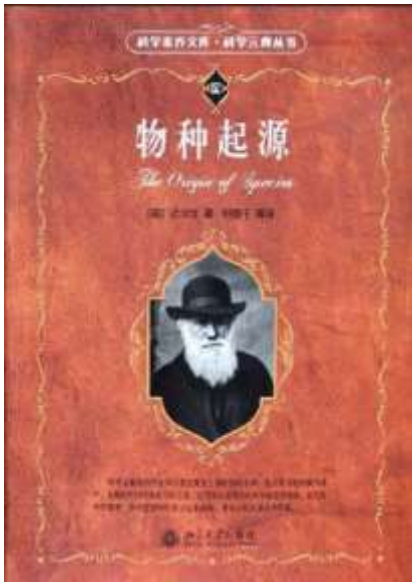
进化论：



拉马克平行进化理论



达尔文生命之树理论，生命分支进化，自然选择



物种渐变猜想	拉马克第一猜想
自然选择猜想	威尔斯-达尔文-华莱士猜想
间断平衡猜想	达尔文-艾/古猜想
生命之树猜想	达尔文猜想

地球上所有生命皆源出于一个或几个共同祖先，沿着38亿年时间长轴不断分支代谢，形成今天这枝繁叶茂的生命大树。天下生命原本一家亲！

----------物种起源

达尔文的旅行考察

19世纪30年代，达尔文乘“贝格尔”号（即“小猎犬”号）舰进行了历时5年的环球航行，对动植物和地质结构等进行了大量的采集和观察，并于1859年出版了《物种起源》这一划时代的著作。 在本书中，达尔文首次提出了进化论的观点，证明物种的演化是通过自然选择和人工选择的方式实现的。 进化论被恩格斯誉为19世纪自然科学的三大发现之一，对后世影响深远。

生命并非神创造，而是不断进化分支、自然选择出现，性状如何从亲本向子代传递？

分子生物学起源之一：

传递遗传学（*Transmission Genetics*）

Transmission Genetics

- Transmission genetics deals with the transmission of traits from parental organisms to their offspring

Note: Chemical composition of genes not known until 1944

- Gene
- Phenotype

Transmission Genetics

Gregor Mendel

Thomas Hunt Morgan

Barbara McClintock

现代遗传学之父-孟德尔

Gregor Mendel.
Um das Jahr 1862.

Mendel & the Garden Peas

- Gregor Mendel, Austrian Monk (1822-1884)
- Educated in a monastery and ordained in 1847
- Failed his exam for teaching certificate
- Studied math and science at University of Vienna
- Returned to monastery for rest of his life
- Began his experiments with the hybrid cultivation of pea plants in 1856

Mendel & the Garden Peas

- In 1866, Mendel performed breeding experiments with garden pea plants and observed consistent, predictable patterns of inheritance.
- From his observations, Mendel developed a number of principles, today referred to as Mendel's Laws of Inheritance.
- His theory was recognized and well accepted 35 years after it’s published when he had passed away for 16 years.

融合遗传:1868年由达尔文提出

- In the 19th century, when both Gregor Mendel and Charles Darwin were alive, "**blending inheritance**" was a popular idea.
- According to blending inheritance, traits from parents "blended" in offspring producing intermediate traits.
- **Example**: The offspring of one short parent and one tall parent would be of medium height. This offspring would pass the new "medium sized" trait to its own offspring. （what is the consequence if this is true）

与融合遗传相悖的孟德尔遗传定律

融合遗传：权威不总是对的

研究对象及性状的选择

种子形状	子叶颜色	种皮颜色	豆荚形状	豆荚颜色	花的位置	茎的高度
 圆滑	 黄色	 灰色	 饱满	 绿色	 叶腋	 高茎
 皱缩	 绿色	 白色	 不饱满	 黄色	 茎顶	 矮茎

豌豆闭花授粉，杂交时不受外界影响，性状明显又稳定。

Mendel's first law, or the principle of segregation (独立分离定律）

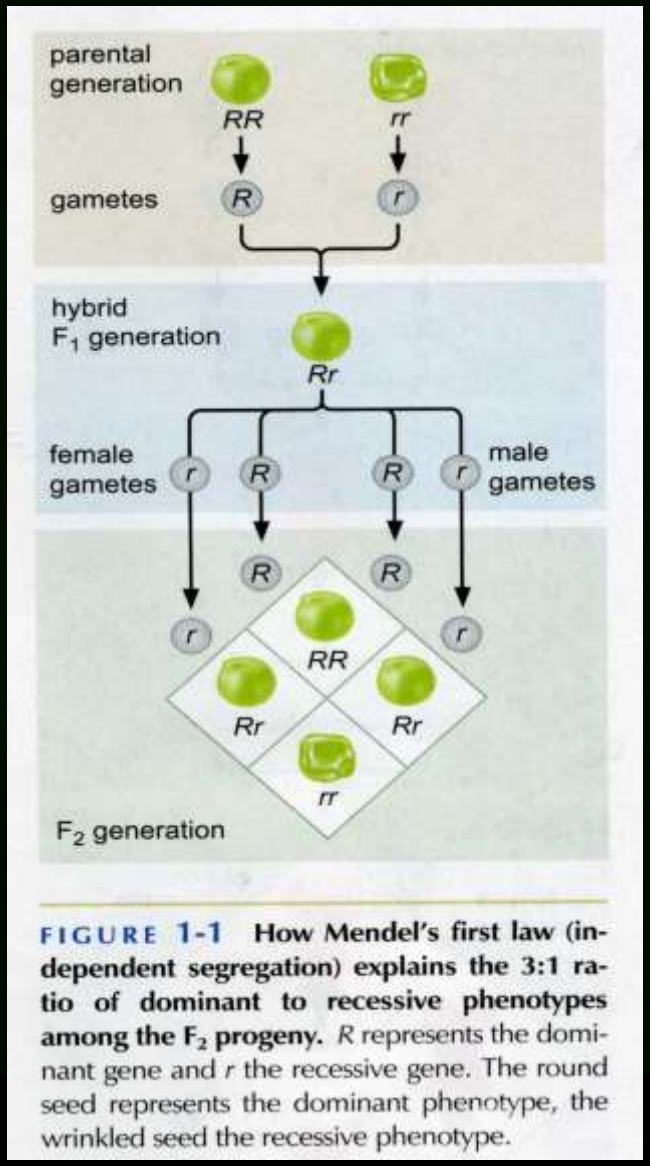
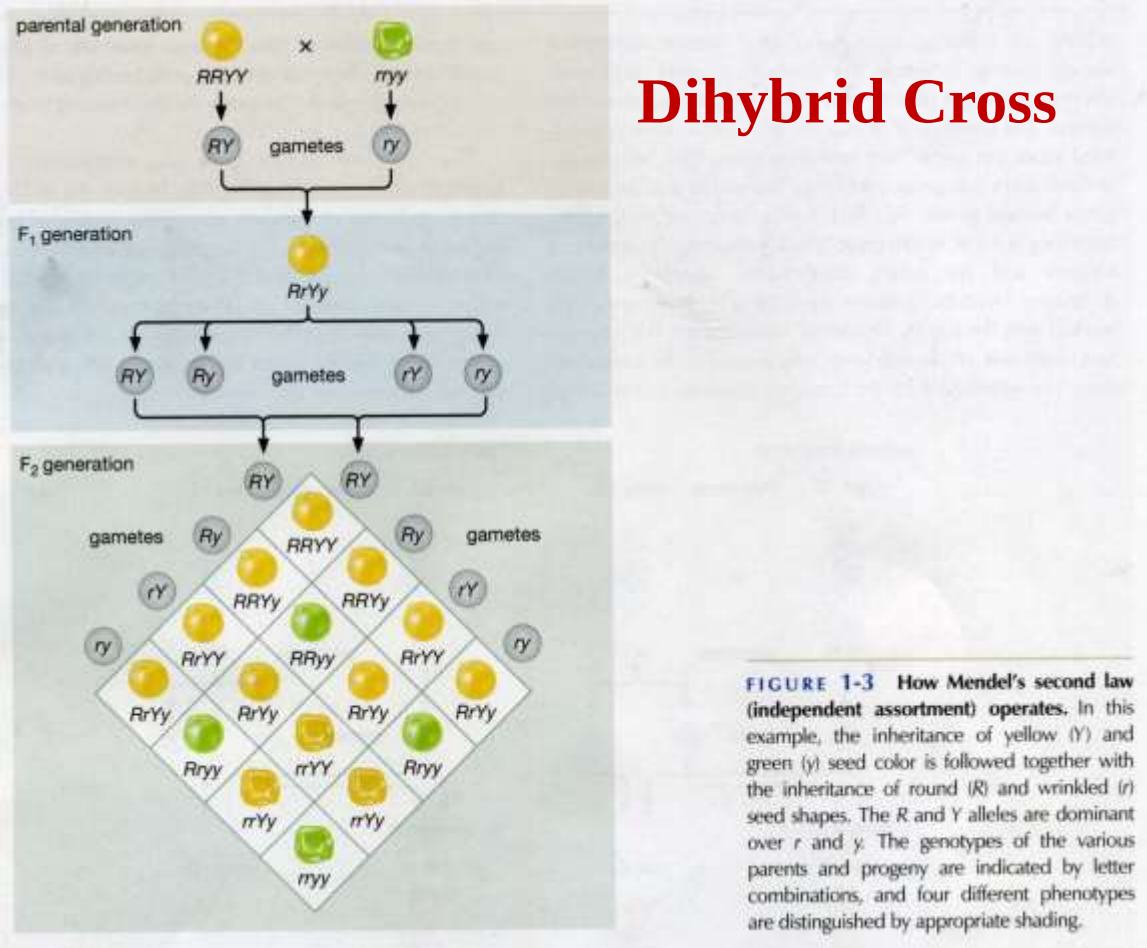


FIGURE 1-1 **How Mendel's first law (independent segregation) explains the 3:1 ratio of dominant to recessive phenotypes among the F_2 progeny.** *R* represents the dominant gene and *r* the recessive gene. The round seed represents the dominant phenotype, the wrinkled seed the recessive phenotype.

During the formation of eggs and sperm, "paired factors" (now known as alleles等位基因, which reside on chromosomes) segregate, or separate.

Mendel's second law
the principle of independent assortment
（独立分配定律）



Dihybrid Cross

FIGURE 1-3 **How Mendel's second law (independent assortment) operates.** In this example, the inheritance of yellow (Y) and green (y) seed color is followed together with the inheritance of round (R) and wrinkled (r) seed shapes. The R and Y alleles are dominant over r and y. The genotypes of the various parents and progeny are indicated by letter combinations, and four different phenotypes are distinguished by appropriate shading.

Each pair of "factors" (now known as alleles) separate and recombine into gametes independently of each other.

孟德尔遗传定律
Mendel's Laws of Inheritance

- A gene can exist in different forms called **alleles**
- One allele can be **dominant** over the other, **recessive**, allele
- The first **filial** generation (F_1) contains offspring of the original parents
- If each parent carries two copies of a gene, the parents are **diploid** for that gene

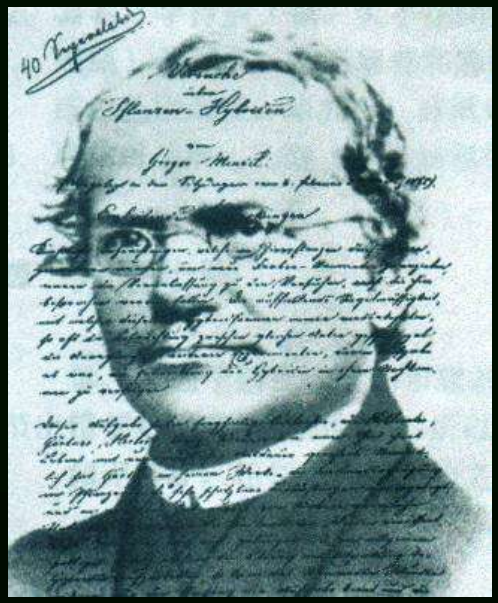
Mendel's Gene Transmission

- **Heterozygotes have one copy of each allele**
- **Parents in 1st mating are homozygotes, having 2 copies of one allele**
- **Sex/Germ cells, or gametes, are haploid, containing only 1 copy of each gene**
- **Heterozygotes produce gametes having either allele**
- **Homozygotes produce gametes having only one allele**

In 1865, Gregor Mendel published *Mendelian inheritance (孟德尔遗传定律).*

遗传就是将来自亲本的各种性状传给子代

遗传性状依靠独立的遗传因子传递给后代；每一个亲本只传递一半的遗传因子给每一个子代, 并自由组合



1822-1884

garden pea

Summary

- Genes can exist in different forms or **alleles.**
- One allele can be dominant over another, so **heterozygotes** having two different alleles of one gene will generally exhibit the characteristic dictated by the **dominant allele.**
- The **recessive** allele **is not lost**; it can still exert its influence when paired with another recessive allele in a homozygote.

豌豆实验豌豆实验发现了遗传学三大基本规律中的两个：

分离定律

自由组合定律

种质学说

孟德尔遗传学定律有悖于达尔文融合遗传假说。达尔文的拥护者一直没闲着。

早在1892年，自然选择的强烈拥护者、德国胚胎学家魏斯曼就提出了「种质学说」，认为生物主管遗传的「种质」与主管营养的「体质」是完全分离的，这已经非常接近孟德尔的分离定律和自由组合定律。

Why other scientists did not know about or uniformly ignored the implications of Mendel's work until 1900?

1900年，三位植物学家：荷兰植物学家德弗里斯、德国植物学家科伦斯、奥地利植物学家丘歇马克通过各自独立的工作，在《德国柏林植物学会》杂志发表3篇植物杂交论文，在论文发表前夕查阅文献，三人几乎同时发现了孟德尔1866年发表的论文——**《植物杂交实验》**，因此证实了孟德尔的结果，并接受了颗粒遗传的特点，所以孟德尔最先发现了遗传的基本规律。

突变论

1901年德弗里斯提出「突变论」，指出物种的形成途径不仅有渐变式，更有大量的突变式。

遗传的染色体理论：
（*Chromosome theory of inheritance*）

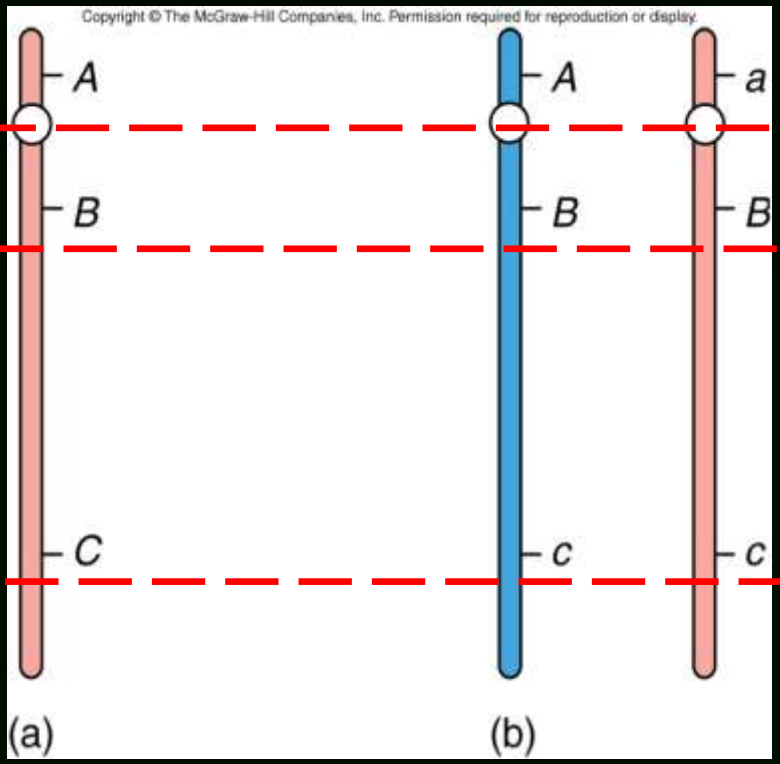
精髓：染色体携带基因

该理论是从遗传学思考问题的新基础

Hypothetical Chromosomes

- Every gene has its place, or **locus**（基因座）, on a chromosome
- **Genotype** is the combination of alleles found in an organism
- **Phenotype** is the visible expression of the genotype
  - **Wild-type** phenotype is the most common or generally accepted standard（Standard Phenotype)
  - **Mutant** alleles are usually recessive

Location of Genes on Chromosome



(a) 每个基因在染色体上都有其位置或基因座（**Locus**）
(b) 二倍体生物，所有染色体（性染色体除外）通常是双份的，大部分基因具有两个拷贝

1928年，摩尔根发表《基因论》，指出原本抽象的概念「基因」是确实存在于细胞的染色体上的，并据此提出了「连锁遗传定律」。从基因层面部分解释了「突变式」的原因。

1933年，摩尔根获诺贝尔奖

常染色体和性染色体

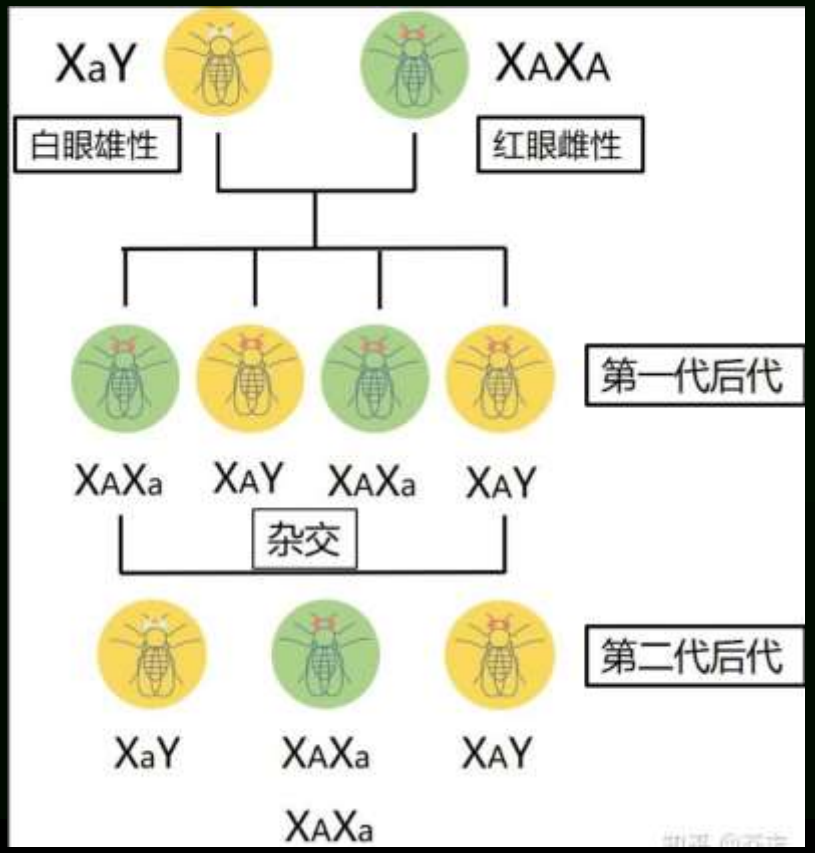
- **Autosomes** occur in pairs in a given individual
- **Sex chromosomes** are identified as X and Y
  - Female has two X chromosomes
  - Male has one X and one Y chromosome

果蝇的性连锁遗传

将红眼果蝇（显性）与白眼果蝇（隐性）杂交后大部分F1代是红眼， 为什麽？

F1代雄性红眼与其红颜姐妹杂交后，产生约1/4雄性白眼，但没有雌性白眼，为什麽？

答案：眼睛颜色表型是性连锁的（Sex-linked），性别和眼色一起传递是因为控制眼睛颜色的形状位于X性染色体。雌性果蝇有两条X染色体，而雄性果蝇含一条X染色体，一条Y染色体。



果蝇为什麽比豌豆更适合实验

个体小

时代周期短

后代数量多

性状也很稳定

遗传重组和遗传定位

Genetic Recombination and Mapping

- In early experiments genes on separate chromosomes behaved independently（分离、自由组合）
- Genes on the same chromosome behaved as if they were linked（遗传连锁）

This genetic linkage is not absolute

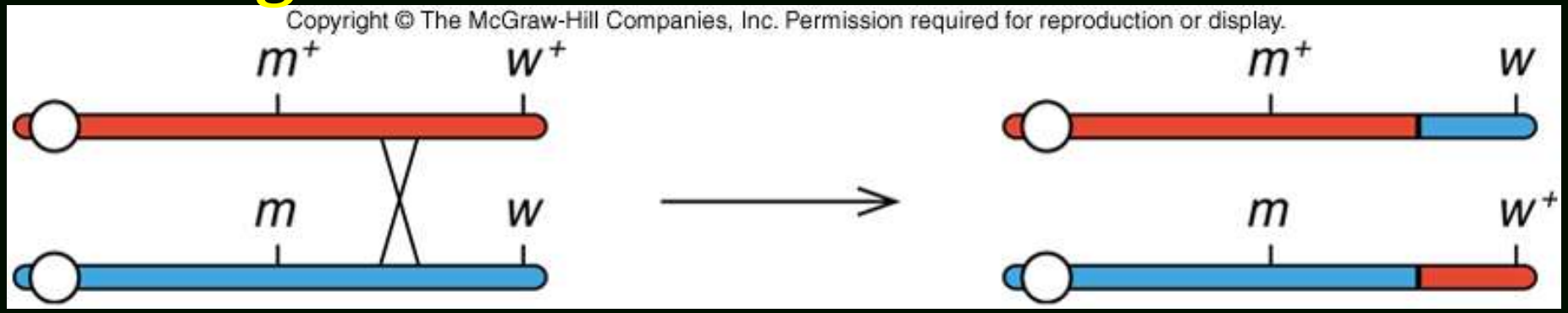
Offspring show new combinations of alleles not seen in the parents when recombination occurs

Why?

答案：重组

Recombination in *Drosophila*

Exchange of Locus on chromosome



染色体1含： $m^+ w^+$
配对染色体含： mw

染色体1含： $m^+ w$
配对染色体含： m w

Recombination-重组

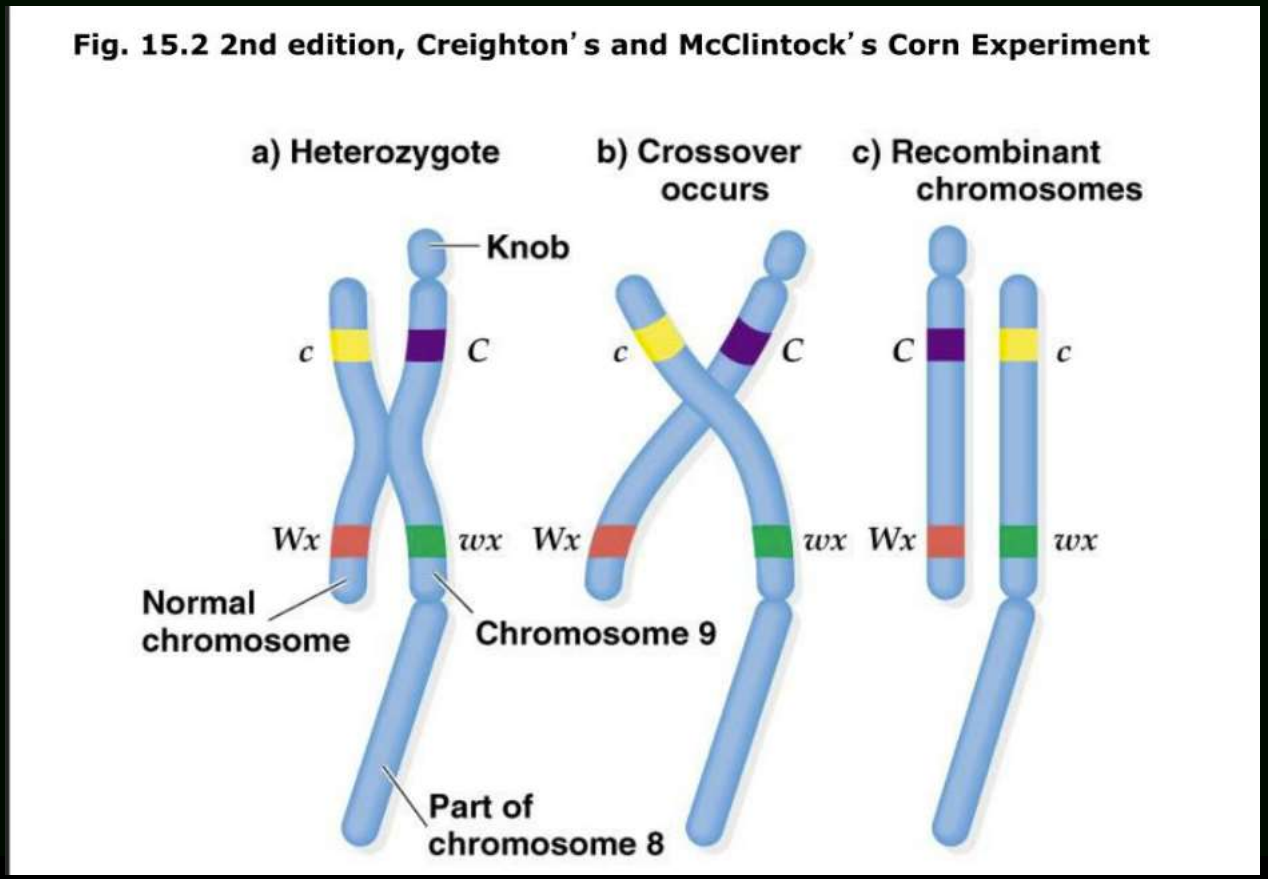
- During **meiosis**, gamete formation, crossing over can occur resulting in the exchange of genes between the two homologous chromosomes
- The result of the **crossing-over** event produces a new combination of alleles
- This process is called **recombination**

遗传作图：Genetic Mapping

- Morgan proposed that **the farther apart** two genes are on a chromosome, the **more likely they are to recombine**
- **A.H.Sturtevant用数学关系拓展了该假说**
- If two loci recombine with a frequency of 1%, they are said to be separated by a map distance of one centimorgan (named for Morgan, 厘摩)
- This mapping observation applies both to bacteria and to eukaryotes

重组的物理学证据：
玉米染色体显微观察

（一端有节，一端长的，伸展的）



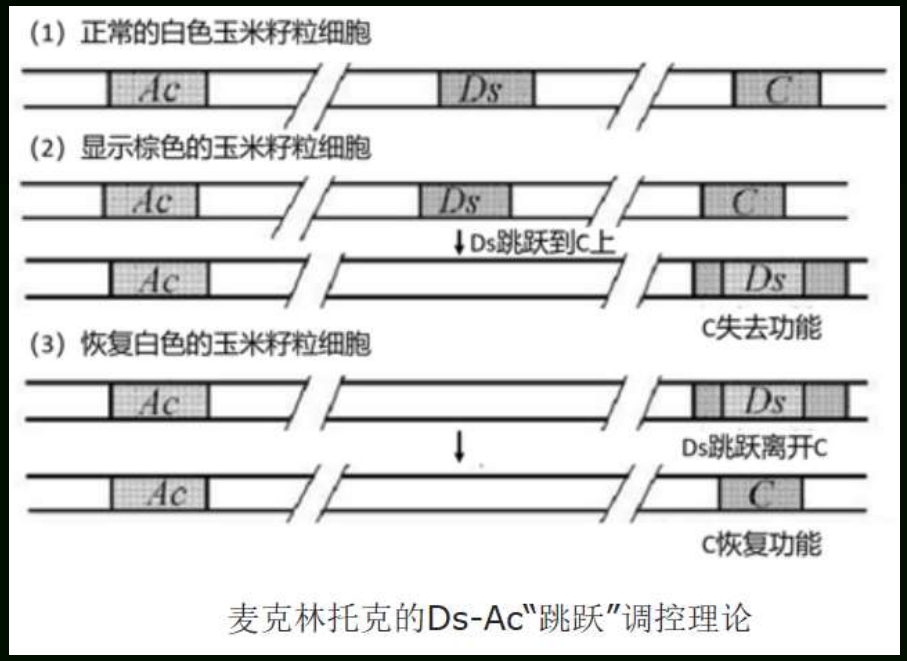
重组的物理学证据：
Physical Evidence for Recombination

- Microscopic examination of **maize chromosome** provided direct physical observation of recombination using easily identifiable features of one chromosome
- Similar observations were made in Drosophila
- Recombination was detected both physically and genetically in both animals and plants

转座子（跳跃基因的发现）

Paul Chomet and *Rob Martienssen*, *Cell*, 2017

从1932年开始，麦克林托克就在印度彩色玉米中观察到了玉米籽粒与叶片的色斑无法稳定遗传的现象，而且色斑的大小和出现的时间也是各有不同。为了找到这个问题的答案，麦克林托克培育出带有某条特定染色体的玉米植物，该染色体的存在会导致隐性棕色表型（bz基因），而该玉米原本具有的染色体编码白色显性表型（无色，C基因）。根据正常的遗传理论，后代应该显示出全白色的籽粒，但是部分籽粒显现出了白色带棕色斑点的性状。



麦克林托克的Ds-Ac"跳跃"调控理论

麦克林托克将意外的变化归因于染色体断裂，使染色体丢失一个基因，她称这种现象为解离，发生在她称为Ds的染色体基因座上，此外，她认为这种断裂是由于“跳跃”活化剂（Ac）引起的，Ac促使Ds"跳跃”，Ds的移动可能导致被插入基因C产生突变而不能正常行使功能，则隐性基因得到优势，将显示为棕色，而Ac控制Ds从突变的C基因处“跳走”又可以恢复C基因的功能，显示白色，所以，玉米籽粒白色背景上棕色斑点的数量取决于籽粒发育期间基因“跳跃”的时间

1951年在冷泉港会议提出基因跳跃理论，1983年获得诺贝尔奖

SUMMARY:

- The chromosome theory of inheritance holds that genes are arranged in linear fashion on chromosomes.
- The reason that certain traits tend to be inherited together is that the genes governing these traits are on the same chromosome.
- However, recombination between two homologous chromosomes during meiosis can scramble the parental alleles to give nonparental combinations.
- The farther apart two genes are on a chromosome, the more likely such recombination between them will occur.

诱发革命并将它引向深纵发展的驱动力是几个伟大的科学猜想以及人们对这些猜想执着的求证。在科学的征程上，人们须有继续前行的勇气和决心，但在崎岖山路上更离不开拉马克、达尔文、孟德尔、摩尔根、麦克林托克等这些「灯塔」的指引。

传递遗传学（Transmission Genetics)

分子遗传学（Molecular Genetics)

Molecular Genetics

- The Discovery of DNA
- The Relationship Between Genes and Proteins
- Activities of Genes

The Discovery of DNA

1869, **Friedrich Miescher**

Discovered nuclein and the major component of nuclein is DNA.

1844-1895

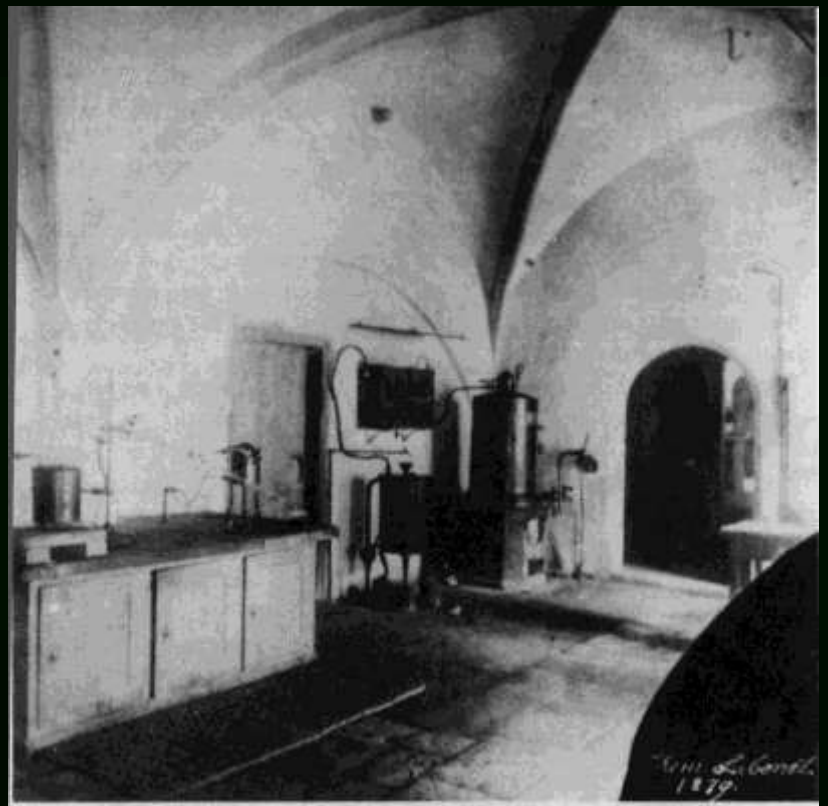


Figure 1.5 The laboratory at Tübingen where Miescher isolated nuclein (courtesy of the University of Tübingen Library, Tübingen, Federal Republic of Germany).

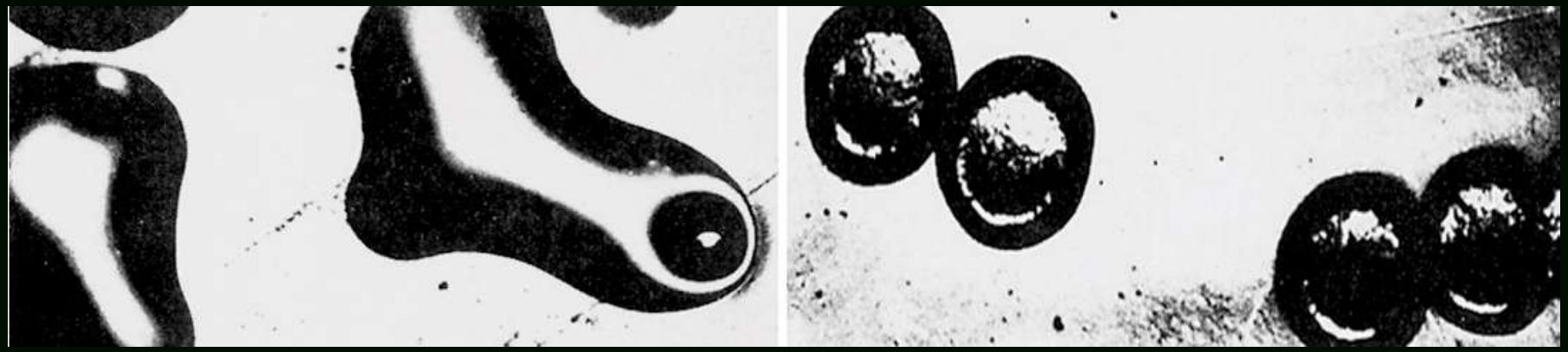
http://www.fmi.ch/members/marilyn.vaccaro/ewww/index2.html

The Discovery of DNA

Historical Background

- **Miescher** isolated nuclei from pus cells (white blood cells) in 1869
  - Found a novel phosphorus-bearing substance = nuclein (核素)
  - **Nuclein** is mostly chromatin, a complex of DNA and chromosomal proteins
- End of 19th century – DNA and RNA separated from proteins
- **Levene, Jacobs**, et al. characterized basic composition of DNA and RNA

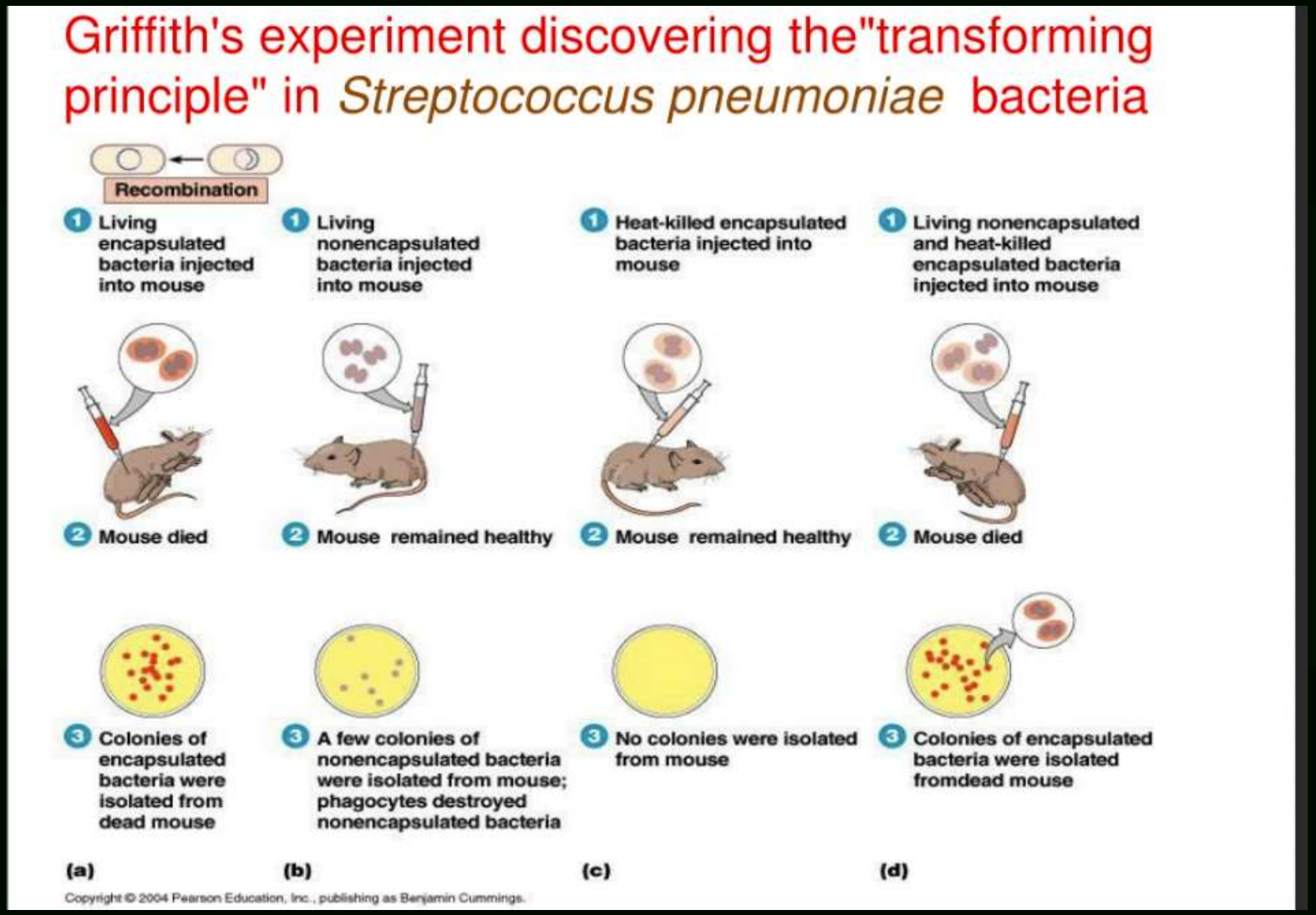
Transformation in Bacteria



Large, glossy, smooth virulent bacteria(S) Small, mottled, rough avirulent bacteria(R)

- Key experiments done by **Frederick Griffith** in 1928
- Observed change in *Streptococcus pneumoniae* （肺炎双球菌）— from virulent (S) smooth colonies where bacterial had capsules, to avirulent (R) rough colonies without capsules

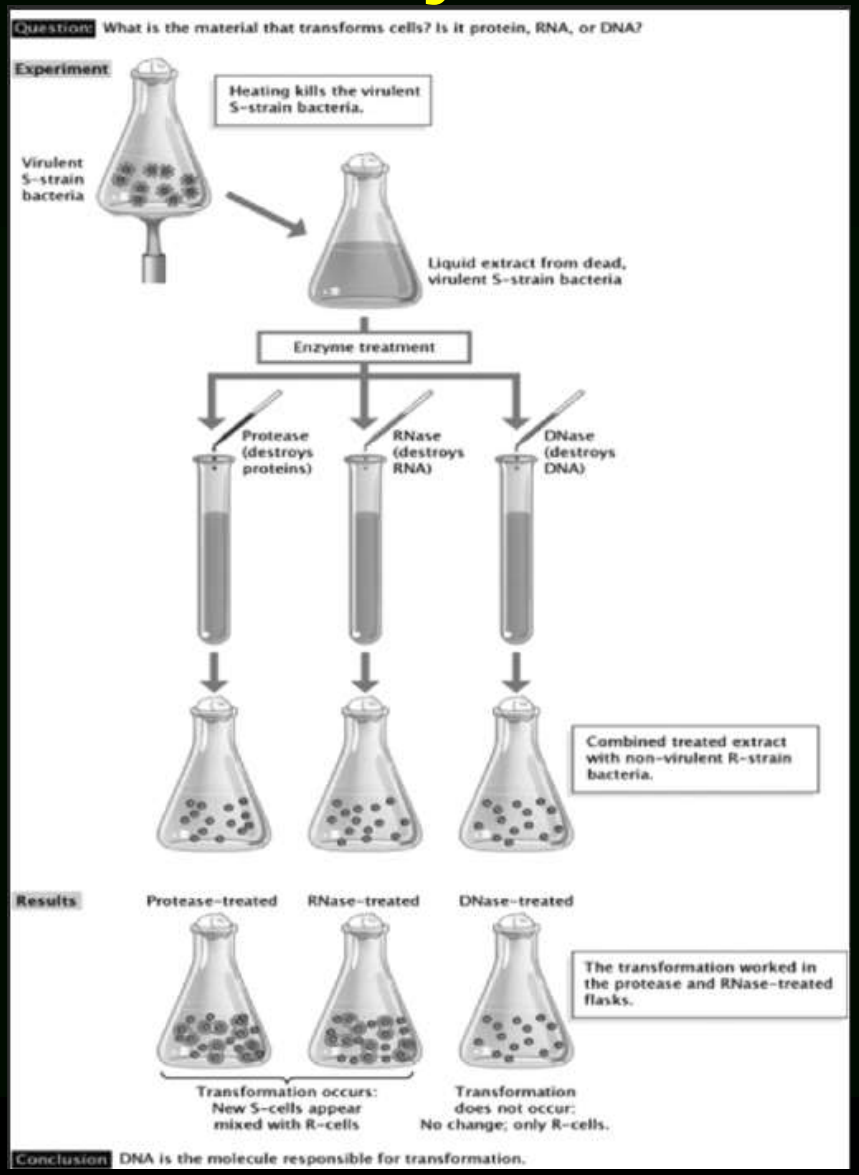
Griffith's Transformation Experiments



Transformation in Bacteria

- Transformation was **not transient**
- The ability to make a capsule and therefore to kill host animals, once conferred on the avirulent bacteria, can **pass to their descendants as a heritable trait**.
- In other words, the avirulent cells somehow gained the gene for virulent during transformation.
- What is the transforming substance ?

1944 by Oswald T. Avery



Removed the protein: no effect

Organic extract

Trypsin

chymotrypsin

Destroy RNA: no effect

RNase

Destroy DNA: Destroy

DNase

DNA Confirmation

- In 1940s geneticists doubted use of DNA as it appeared to be monotonous repeats of 4 bases
- By 1953 **Watson & Crick** published the double-helical model of DNA structure and **Chargaff** had shown that the 4 bases were not present in equal proportions
- **Hershey and Chase** demonstrated that bacteriophage infection comes from DNA

Viral Genes Are Also Nucleic Acids

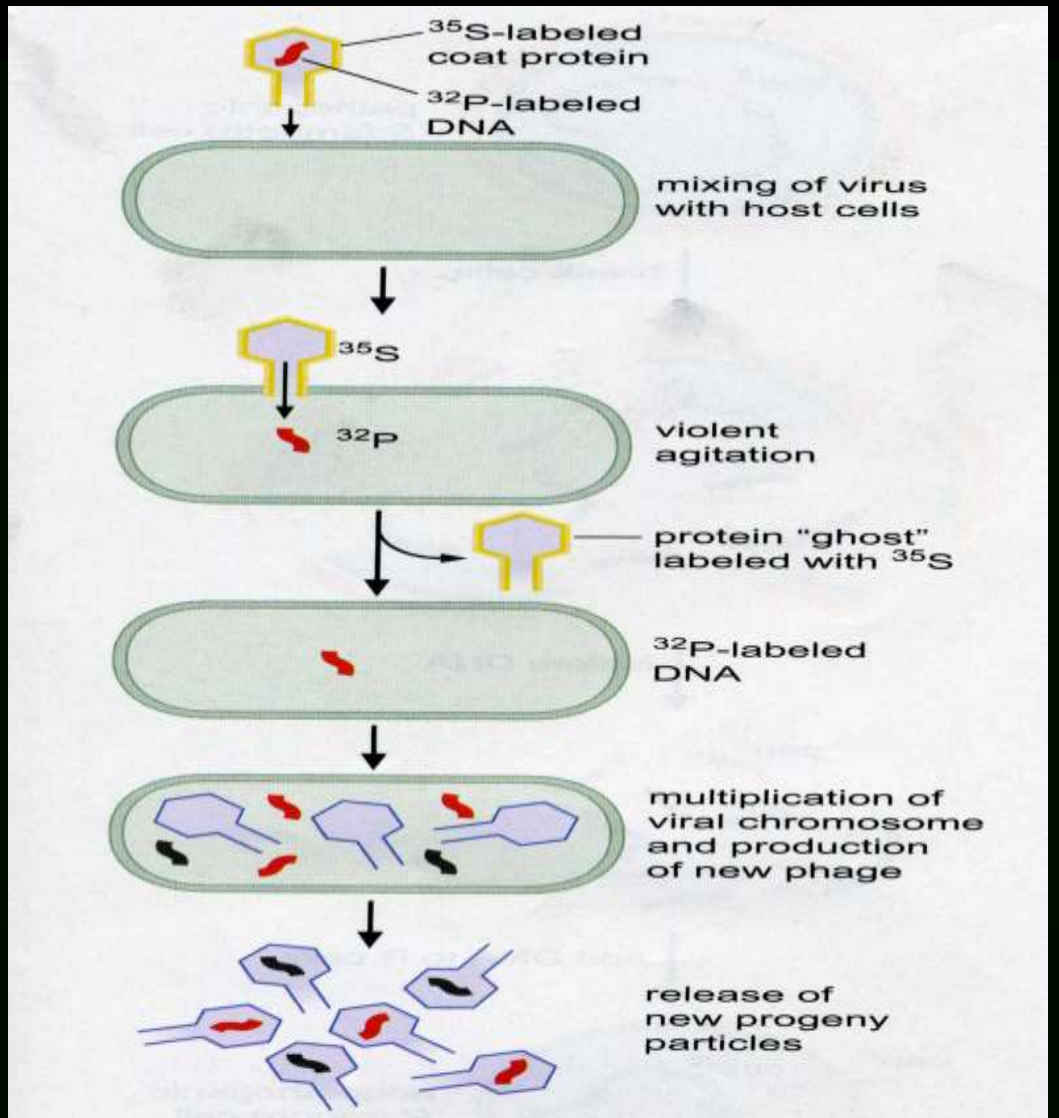


FIGURE 2-3 Demonstration that only the DNA component of the bacteriophage T2 carries the genetic information and that the protein coat serves only as a protective shell.

Summary

- Genes are made of nucleic acid, usually DNA
- Some simple genetic systems such as viruses have RNA genes

2、Molecular Genetics

- The Discovery of DNA
- The Relationship Between Genes and Proteins
- Activities of Genes

The Relationship between Genes and Proteins

Garrod提出尿黑酸症可能由基因突变/缺陷引起，导致患者体内某条生化途径中间产物不正常积累，这是由于将其转化为下一产物的酶缺失或失活。因此提出“一个基因负责产生一种酶的假说”

Archibald Garrod

Garrod, Archibald E. 1902. The Incidence of Alkaptonuria: A Study in Chemical Individuality. *Lancet*, vol. ii, pp. 1616-1620.

THE INCIDENCE OF ALKAPTONURIA:
A STUDY IN CHEMICAL INDIVIDUALITY

ARCHIBALD E. GARROD

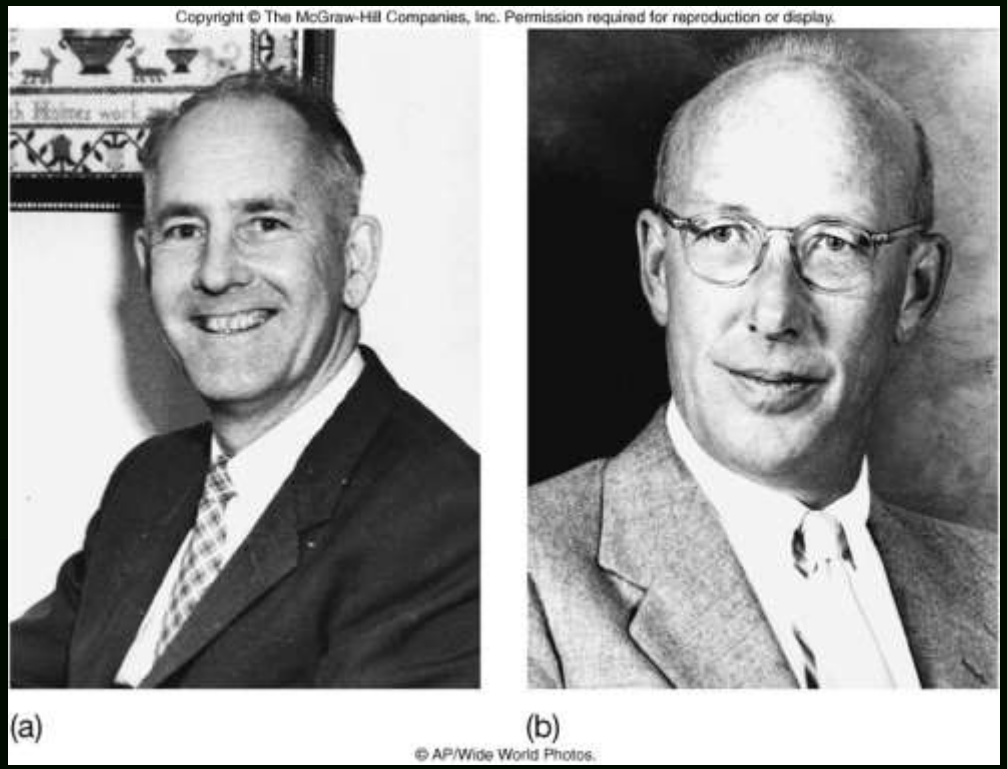
Physician to the Hospital for Sick Children, Great Ormondstreet, Demonstrator of Chemical Pathology at St. Bartholomew's Hospital

ALL THE MORE RECENT WORK on alkaptonuria has tended to show that the constant feature of that condition is the excretion of

病因：常染色体隐性遗传病，先天性尿黑酸氧化酶缺乏，由酪氨酸分解而来的尿黑酸不能进一步分解为乙酰乙酸，过多的尿黑酸由尿排出，空气中氧化为黑色。酪氨酸可在酪氨酸转氨酶的催化下，生成对羟苯丙酮酸，再生成尿黑酸后，进一步转变成乙酰乙酸和延胡索酸，二者分别参加糖代谢和脂代谢. 缺乏尿黑酸氧化酶，尿黑酸不能氧化而自尿中排出，使尿液呈黑色，称尿黑酸症，是先天性代谢缺陷症。

症状：病儿出生后一旦摄取奶，尿中便会排出尿黑酸，排出量与摄取的酪氨酸和苯丙氨酸量成正比。新鲜尿液颜色正常，暴露于空气中，或使其变成碱性时，变成黑色。因为尿布染黑方引起注意。在小儿期除尿黑酸外，并无其他症状。20岁后，由于被氧化的尿黑酸长期沉积于结缔组织中，可导致巩膜，耳郭、鼻、额部变为褐色或蓝黑色，出现揭黄病。沉着于关节的黑色素可引起骨关节炎。可引起循环功能不全而导致病人死亡。

Beadle 和Tatum证实“一个基因负责产生一种酶的假说”



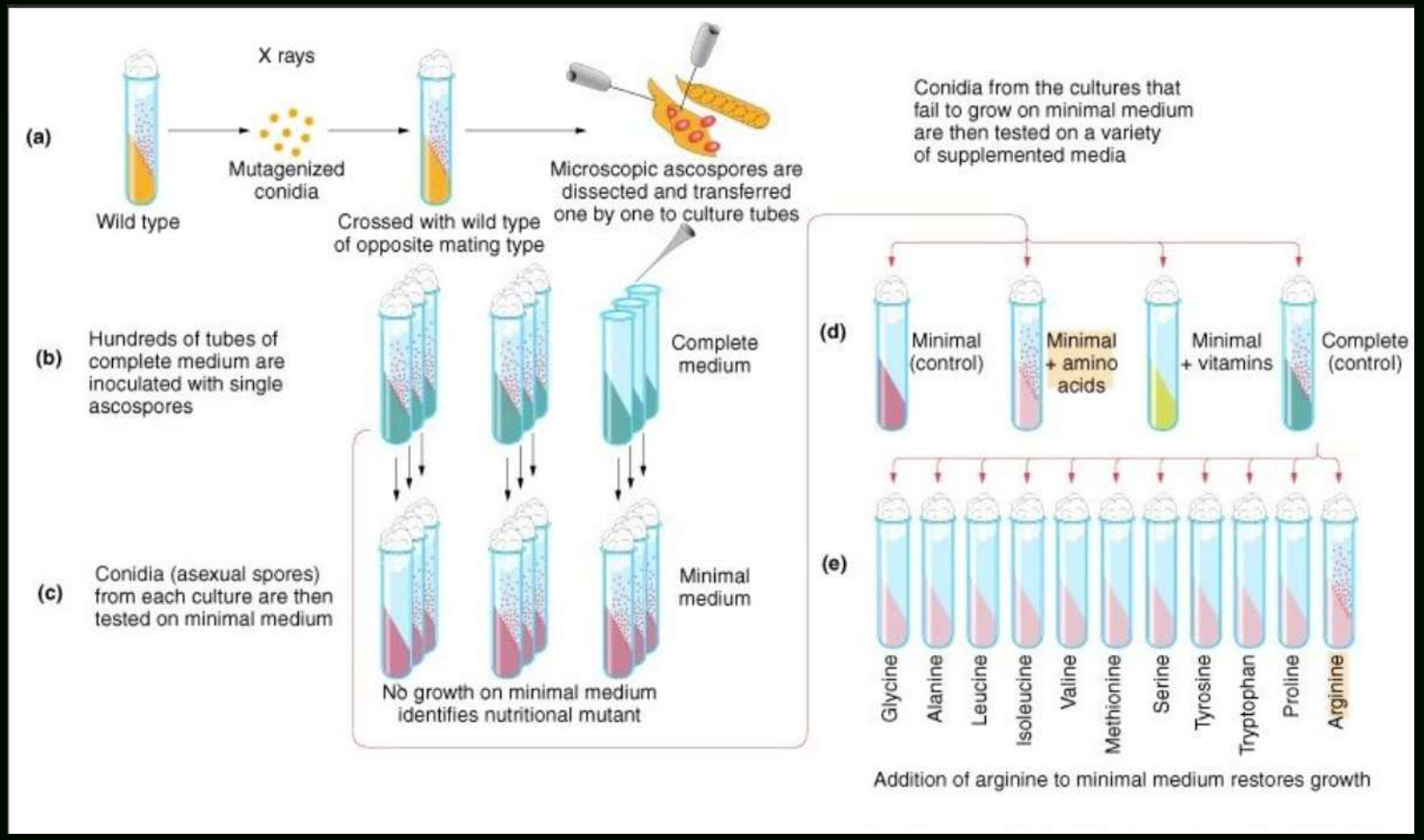
George Beadle E.L.Tatum

In 1902, Archibald Garrod described the inherited disorder alkaptonuria as an "inborn error of metabolism." He proposed that a gene mutation causes a specific defect in the biochemical pathway for eliminating liquid wastes. The phenotype of the disease – dark urine – is a reflection of this error.

This hypothesis was rigorously proven in 1941 by George Beadle and Edward Tatum, using the simple bread mold *Neurospora*. First, they found that molds exposed to radiation lose the ability to produce essential nutrients, and this slowed, even stopped the growth of the mold. Then, they found that growth can be restored by providing the mutated mold with a specific supplement. They reasoned that each mutation must inactivate the enzyme (protein) needed to synthesize the nutrient. Thus, one gene carries the directions for making one protein.

证实“一个基因负责产生一种酶的假说”实验：

粉色面包霉----诱变剂引入突变----由缺陷表型追踪到相关生化途径----推论到某个酶的缺陷----加入缺陷酶的代谢产物----挽救缺陷



一个基因一个酶假说：

适用于原核和低等生物

不完全适用于真核生物：

1.一个真核生物基因可通过选择性剪接产生不同的多肽或蛋白

2.多肽或蛋白不一定都具有酶的特性

3.基因的产物也可以是功能性RNA

4.有些酶由多个亚基组成，需要由不同的基因编码

。。。。。。

2、Molecular Genetics

- The Discovery of DNA
- The Relationship Between Genes and Proteins
- Activities of Genes

Activities of Genes

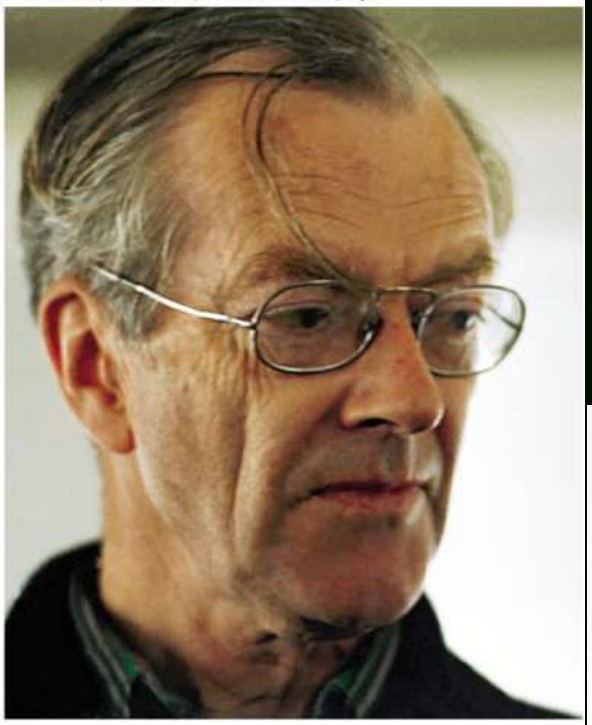
Genes perform three major roles

- Replicated faithfully
- Direct the production of RNAs and proteins
- Accumulate mutations thereby allowing evolution

DNA X射线衍射数据

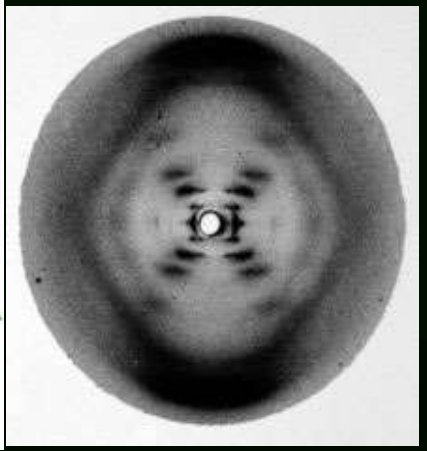
(a)

© From The Double Helix by James D. Watson, 1968, Atheneum Press, NY. © Cold Spring Harbor Laboratory Archives.



(b)

© Courtesy Professor M. H. F. Wilkins, Biophysics Dept., King's College, London.

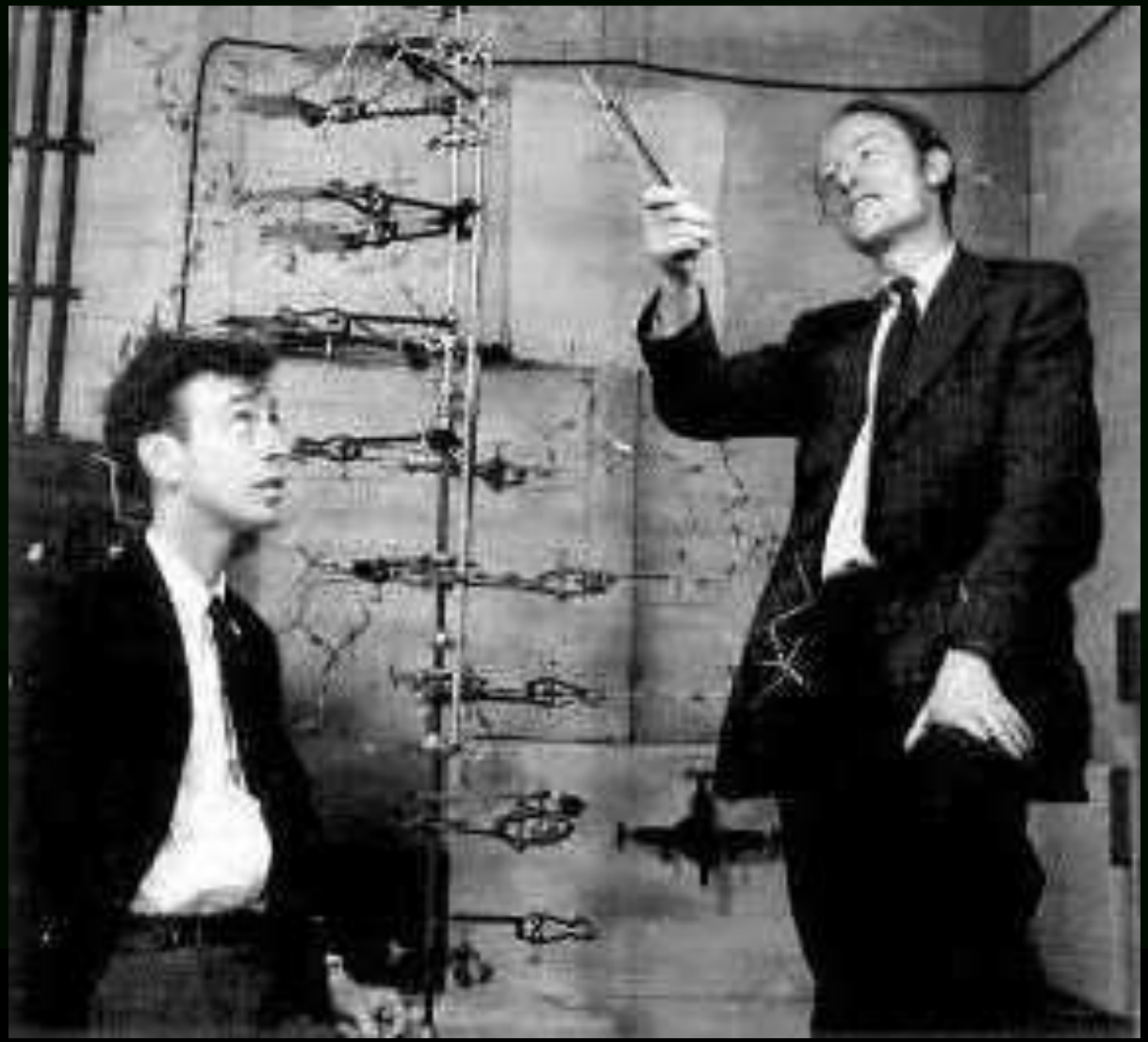


Rosalind Franklin **Maurice Wilkins**

Collect x-ray diffraction data

The Double Helix (1953)

The foundation of molecular biology



Francis H. Crick

James D. Watson

DNA Replication
碱基配对与半保留复制

- **Franklin and Wilkins produced x-ray diffraction data on DNA, Watson and Crick proposed that DNA is double helix**
  - **Two DNA strands wound around each other**
  - **Strands are complementary – know the sequence of one, automatically know the sequence of the other(碱基配对)**

- **Semiconservative replication keeps one strand of the parental double helix conserved in each of the daughter double helices(半保留复制)**

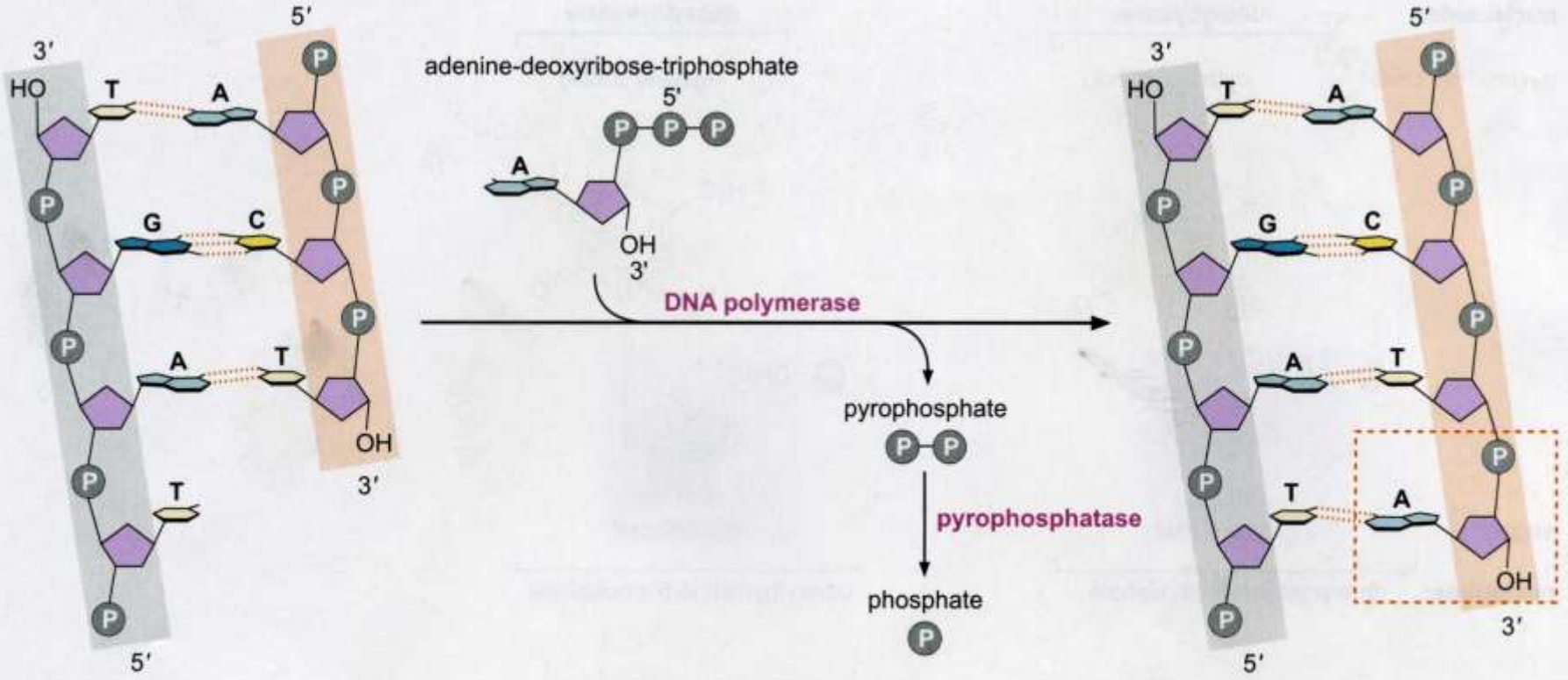


FIGURE 2-8 Enzymatic synthesis of a DNA chain catalyzed by DNA polymerase I. This image shows the addition of a nucleotide to a growing DNA strand as catalyzed by DNA polymerase. Although the DNA polymerase can catalyze DNA synthesis by itself, in the cell the released pyrophosphate molecule is rapidly converted to two phosphates by an enzyme called pyrophosphatase, making the forward reaction of nucleotide addition even more favorable.

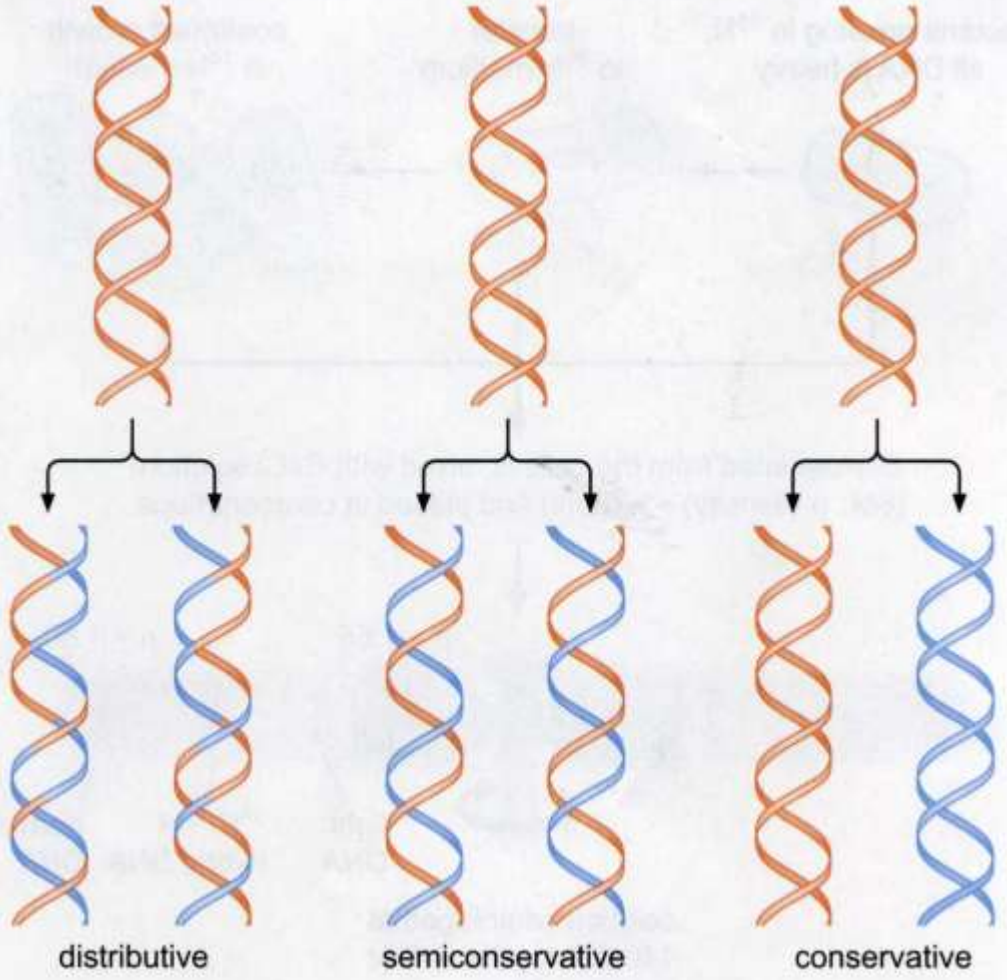


FIGURE 2-10 Three possible mechanisms for DNA replication. When the structure of DNA was discovered, several models were proposed to explain how it was replicated; three are illustrated here. The experiments proposed by Meselson and Stahl clearly distinguished among these models, demonstrating that DNA was replicated semiconservatively.

Meselson和Stahl在细菌中证明了DNA复制遵守半保留方式

(a) © Courtesy Dr. Matthew Meselson.

(b) © Cold Spring Harbor Laboratory Archives.

Matthew Meselson **Franklin Stahl**

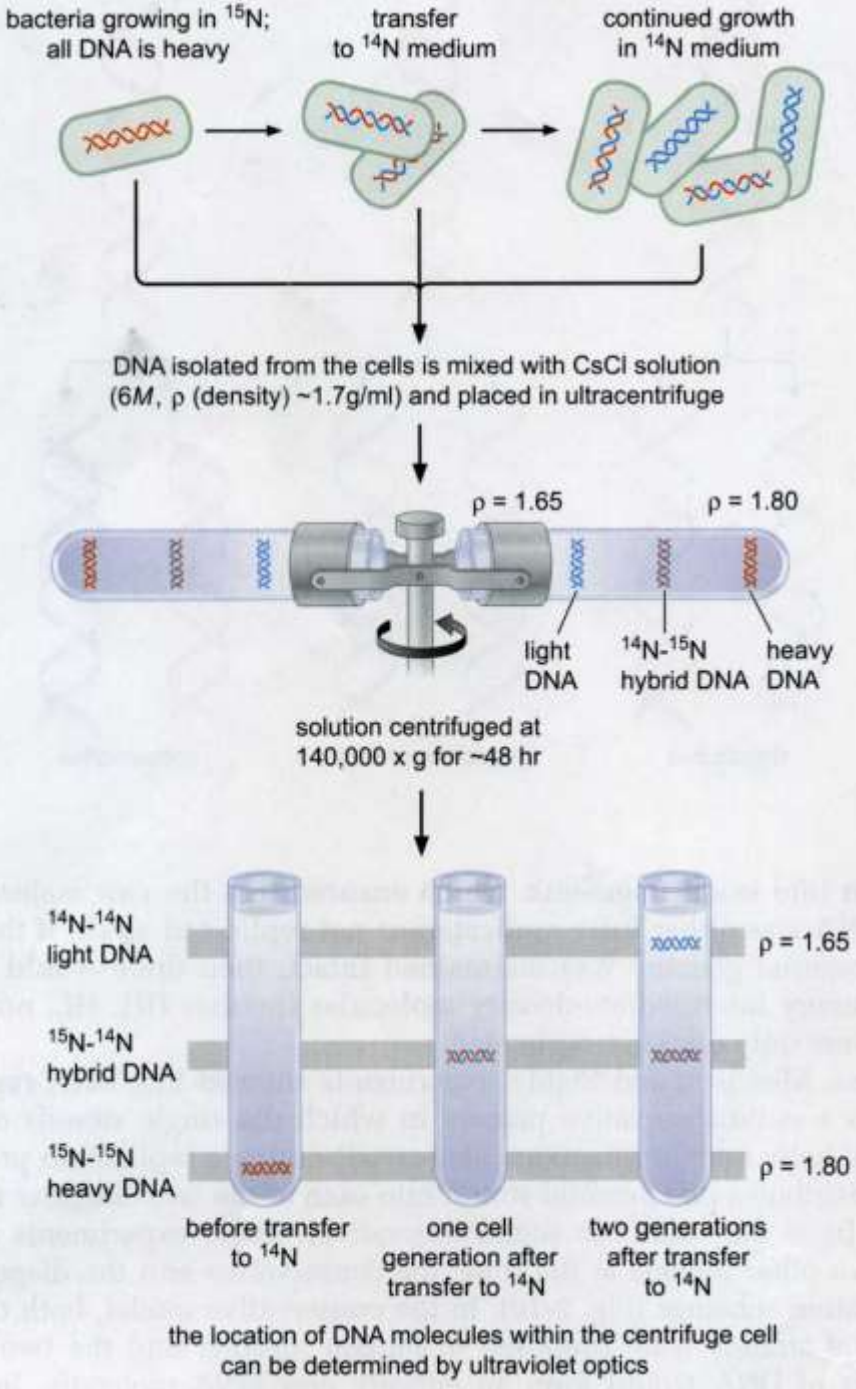


FIGURE 2-9 Use of a cesium chloride (CsCl) density gradient to demonstrate the separation of complementary strands during DNA replication.

Genes Direct the Production of Polypeptides(基因指导多肽的产生）

- **Gene expression** is the process by which a gene product is made
- Two steps are required
  - **Transcription**: copy of DNA is transcribed into RNA
  - **Translation**: the RNA copy is read or translated to assemble a protein
  - **Codon**: a sequence of 3 nucleic acid bases that stand for one amino acid(64个密码子，其中3个终止密码子）

双螺旋DNA含有的遗传信息怎样表达？？

1960年，Jacob和Meselson确定了蛋白质是在细胞质的核糖体上组装的，细胞核信息到了细胞质----不是DNA，提出mRNA假说。1964年，Sydney Brenner使用实验证明了mRNA假说是正确的。

放射性35S 加入到细菌的培养基--以追踪蛋白质的合成--------结果发现同位素标记的新合成的蛋白质与核糖体结合。

35S短暂标记（脉冲），然后使用大量的32S进行追踪，发现与核糖体结合的放射性存留的时间很短。失去的35S标记出现在细胞的可溶性蛋白上。这说明蛋白质是在含有RNA的核糖体上从氨基酸合成而来，一旦合成完成以后，就从核糖体上释放出来。

会不会是核糖体里面的rRNA作为翻译的模板呢？然而，这种可能性很快就被排除，因为他们发现核糖体是完全一样的。

DNA在细胞核里，而遗传信息最后的表达发生在细胞质的核糖体。那么，在细胞内肯定存在其他的成分充当遗传信息传递的载体。1961年，Jacob和Jacques Monod 提出了信使RNA假说，认为细胞内肯定存在一种特殊的RNA是直接从DNA上合成的，它们的序列与DNA上的基因序列互补，然后被运输到细胞质为蛋白质合成提供模板。在一种蛋白质合成结束以后，它的mRNA将离开核糖体，为其他的mRNAs“让路”。

mRNA的假说很快得到了Brenner、Jacob和Meselson的实验确认。他们使用噬菌体感染大肠杆菌，发现感染后不久，就有一种病毒特异性的RNA被合成并很快和细菌内本来存在的含有细菌rRNA的核糖体结合。但这种新的病毒RNA并不是核糖体的永久性成分，而只与核糖体短暂结合。这正是假说中预测的mRNA 分子。

密码子的发现和破译

奥地利物理学家施勒丁格(E．Schrodinger，1944)最早提出遗传密码概念
美国物理学家G．Gamov通过推算提出了三联体密码子的概念，并且进一步推论一种氨基酸可能不止有一个密码子。
克里克(Crick)、布伦纳(S．Brenner)等人以T4噬菌体作为主要研究材料，证实了三联体密码子决定20种不同的氨基酸。

马太(Matthaei)和尼伦伯格(Nirenberg) 破译密码子。
大肠杆菌磨碎制成无细胞提取液，其中含有蛋白质合成所必须的各种酶和氨基酸，然后装入试管，加入少量ATP和人工合成的聚尿嘧啶核苷酸，结果合成的肽链完全是由Phe连接起来的。这一实验说明，Phe的密码子一定是UUU。用同样的方法，得知Pro的密码子是CCC、Lys的密码子是AAA等。

随着技术的改进，以后又人工合成了6种不同的mRNA多聚体，每个多聚体只含有2个碱基，用它们作模板进行蛋白质合成实验。结果表明，在合成的肽链中一种氨基酸和另一种氨基酸的比例决定于上述的碱基比例。例如，用70％的U和30％的A合成RNA，U和A是自由排列的，UUU顺序的三联体的机率是0.7×0.7×0.7≈0.34，即有34％的三联体是UUU。而三联体UUA的机率是0.7×0.7×0.3≈0.15，即15％的三联体是UUA。用上述比例合成的RNA作模板，进行蛋白质的合成。结果发现了30％的聚Phe链和15％的聚Leu链。证明UUU是Phe密码子，而UUA则是Leu的密码子。

Genes Accumulate Mutations

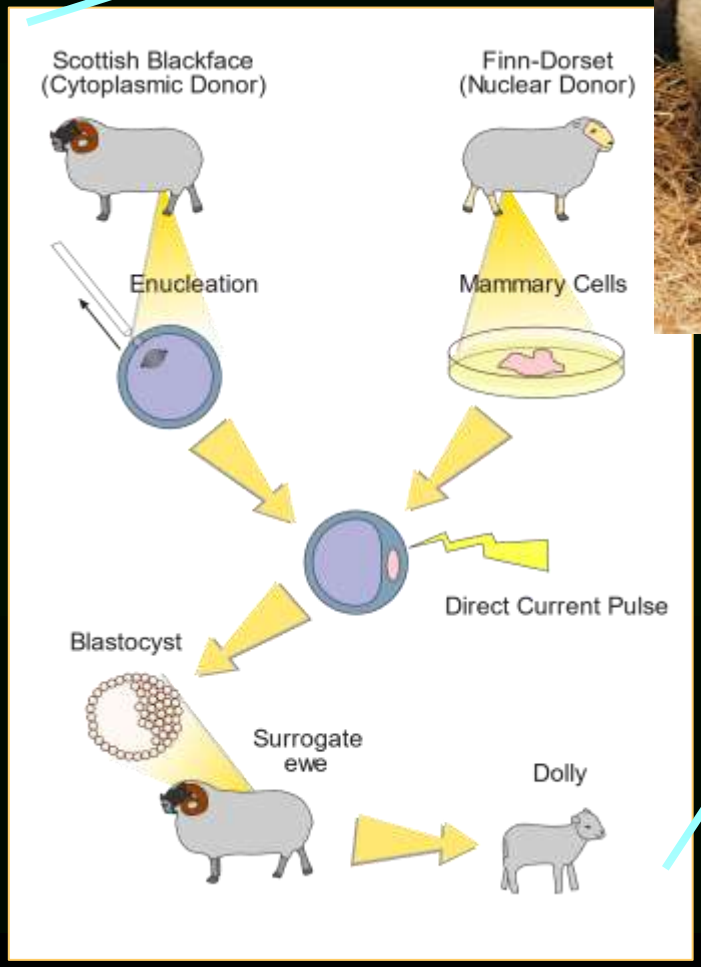
Genes change in several ways

- **Change** one base to another
- **Deletions** of one base up to a large segment
- **Insertions** of one base up to a large segment
- As the change is more drastic, it is more likely that the gene or genes involved will be totally inactivated

Modern Molecular Biology

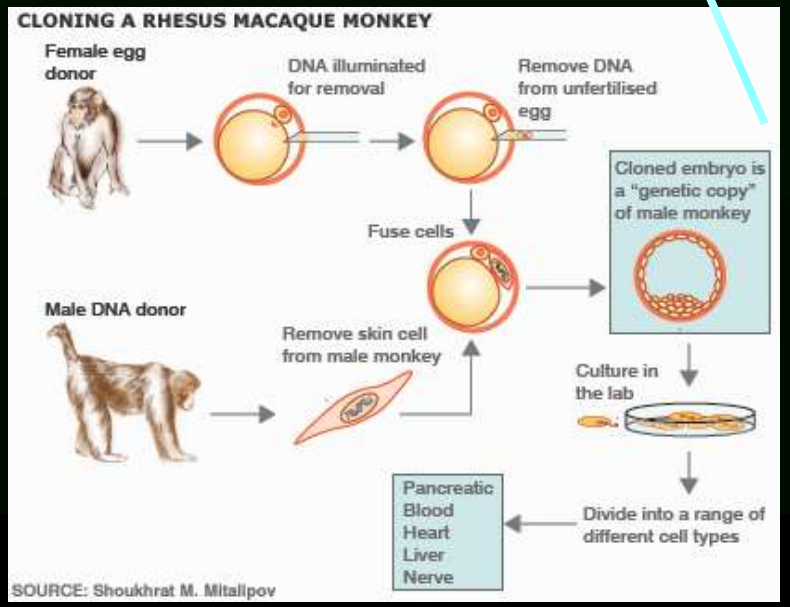
In 1997, lan Wilmut and colleagues cloned a sheep (Dolly) from an adult sheep udder cell.

Cloning of Life



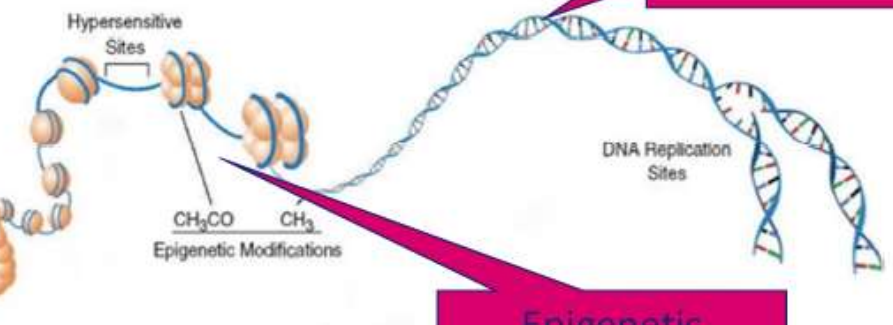
Dolly

“中中”与“华华”



Modern Molecular Biology

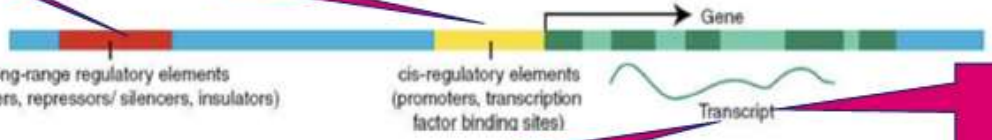
Transcriptional regulatory elements



DNA Methylation

Epigenetic structure (chromatin)

Gene/Protein Interactions



Gene Expression

Small regulatory RNA (eg microRNA)

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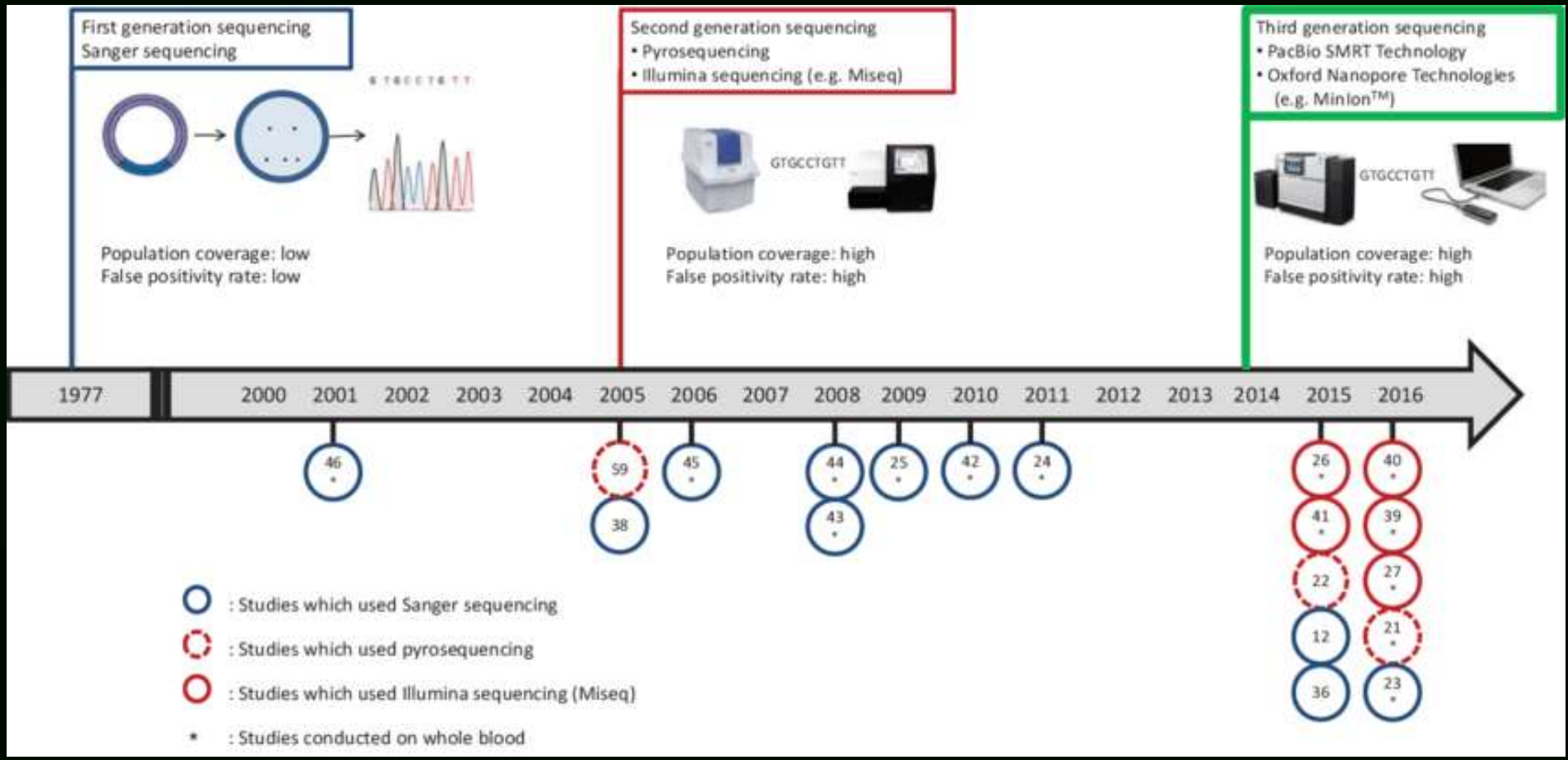
Development of Genome Sequencing Technologies



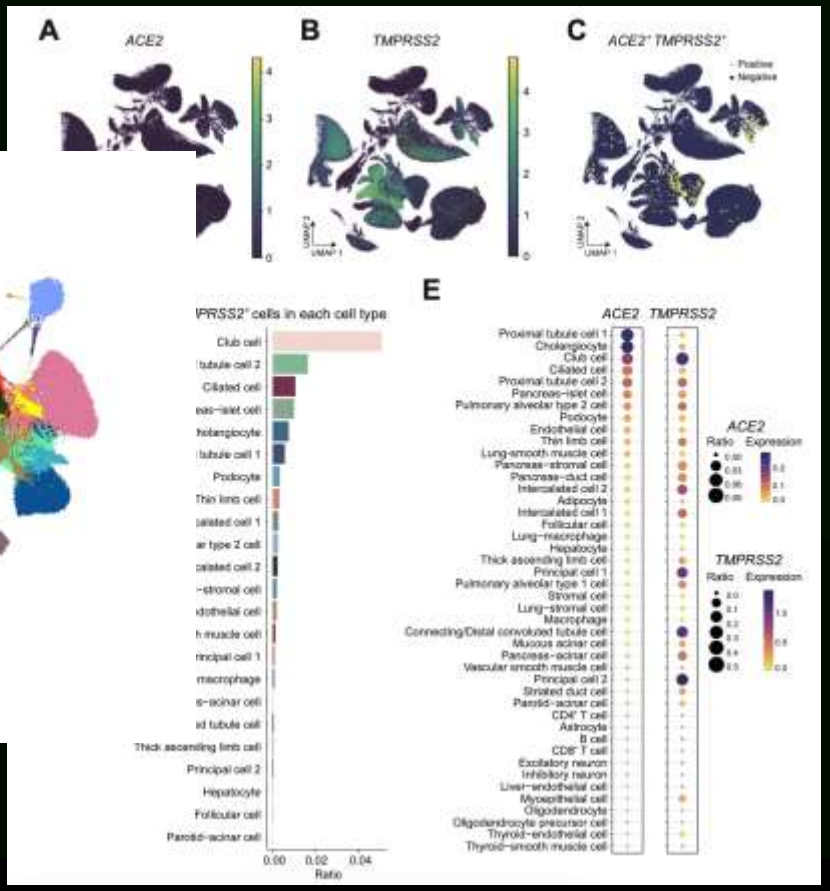
Modern Molecular Biology

人类基因组计划

人类细胞图谱计划

HUMAN CELL ATLAS

新冠病毒受体表达图谱



Modern Molecular Biology

Genome editing:
2011 method of the Year (Nature Methods)

CRISPR/Cas9:
2015 Breakthrough of year (Science)

A market of Billion in Medicine & Agriculture

CARIBOU BIOSCIENCES — Therapeutics Agricultural Biotech

MONSANTO — Agricultural Biotech

editas MEDICINE — Eye Disease

CRISPR THERAPEUTICS — Hematologic Disease Cancer Immune Therapy

Intellia THERAPEUTICS — Liver/Eye/Muscle Disorders

Beam THERAPEUTICS — Base Editing Therapy

课后作业：

生命进化过程中。最先出现的传递信息的大分子物质是什么？

蛋白质?

DNA?

RNA?

其它早被淘汰掉的生物分子？