第三章、DNA复制

第一节：DNA复制的一般特征

吴建新
华南师范大学

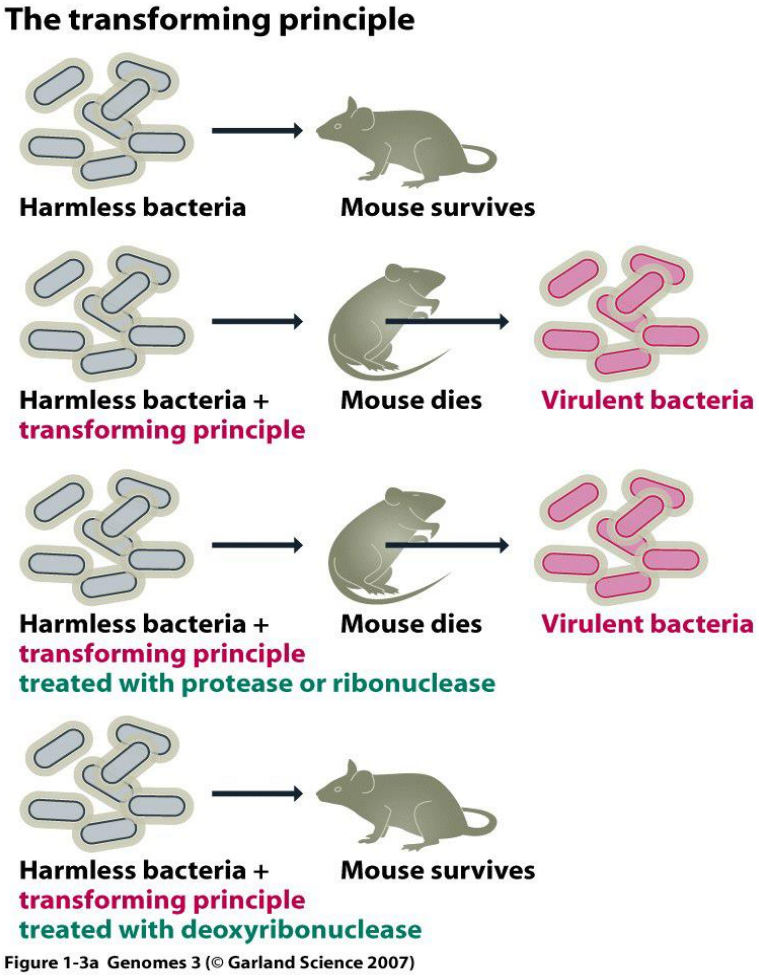


Figure 1-3a Genomes 3 (© Garland Science 2007)

- Oswald Avery(美国)：肺炎链球菌转化实验（1944年）发现转化要素（DNA）是主要的遗传物质。
- 第一次发现遗传物质是DNA，而不是蛋白质。

DNA复制

第一节：DNA复制的一般特征
第二节：DNA复制的过程
第三节：避免5‘端缩短的方式
第四节：DNA复制的模式及调控

DNA双螺旋模型（1953）

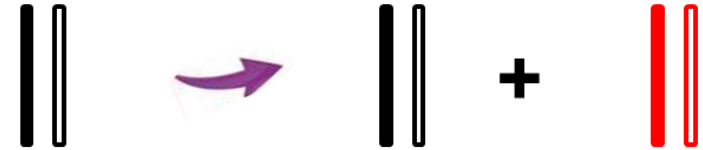
James Watson

Francis Crick

第一节：DNA复制的一般特征

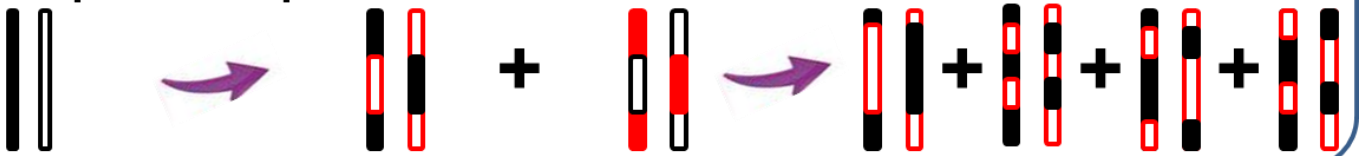
1.1 复制模型

全保留模式
（conservative replication）

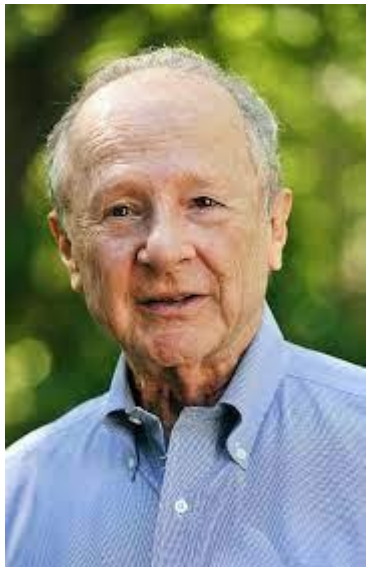


半保留模式
（semiconservative replication）

随机分散保留模式
（random dispersive replication）



Meselson-Stahl experiment



Matthew Meselson Franklin William Stahl

*THE REPLICATION OF DNA IN ESCHERICHIA COLI**

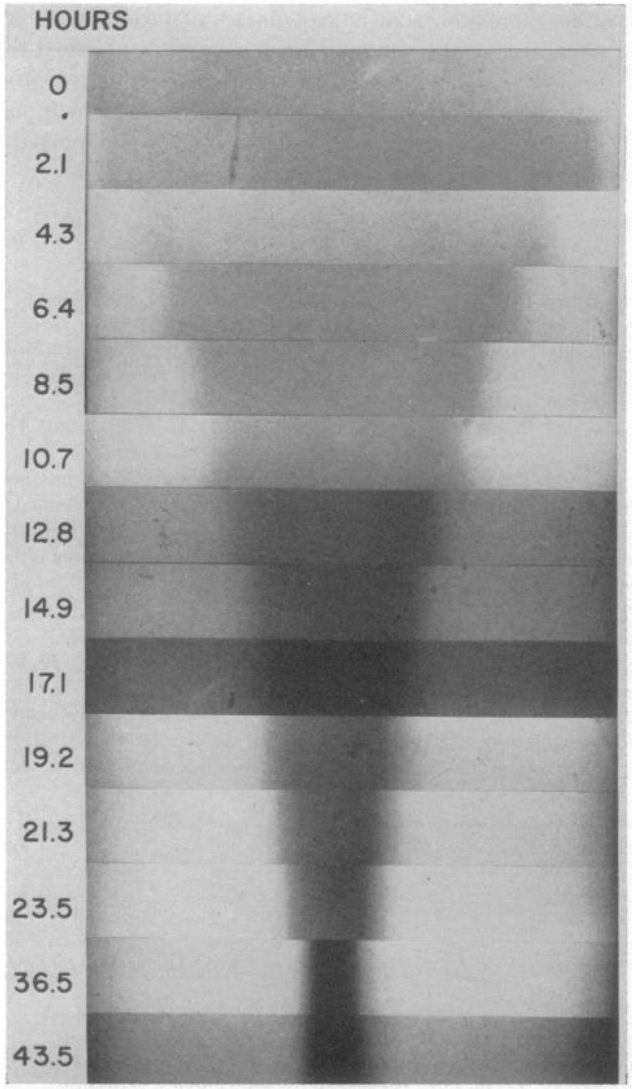
By Matthew Meselson and Franklin W. Stahl

GATES AND CRELLIN LABORATORIES OF CHEMISTRY,† AND NORMAN W. CHURCH LABORATORY OF CHEMICAL BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA

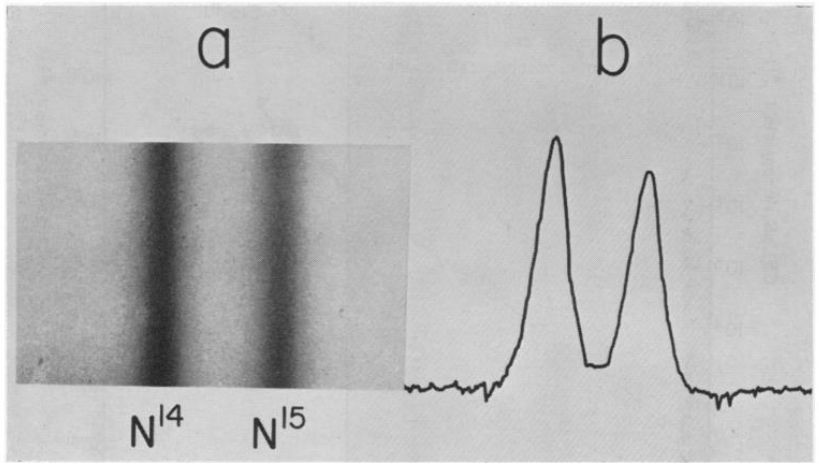
Communicated by Max Delbrück, May 14, 1958

Introduction.—Studies of bacterial transformation and bacteriaphage infection[1–5] strongly indicate that deoxyribonucleic acid (DNA) can carry and transmit hereditary information and can direct its own replication. Hypotheses for the mechanism of DNA replication differ in the predictions they make concerning the distribution among progeny molecules of atoms derived from parental molecules.[6]

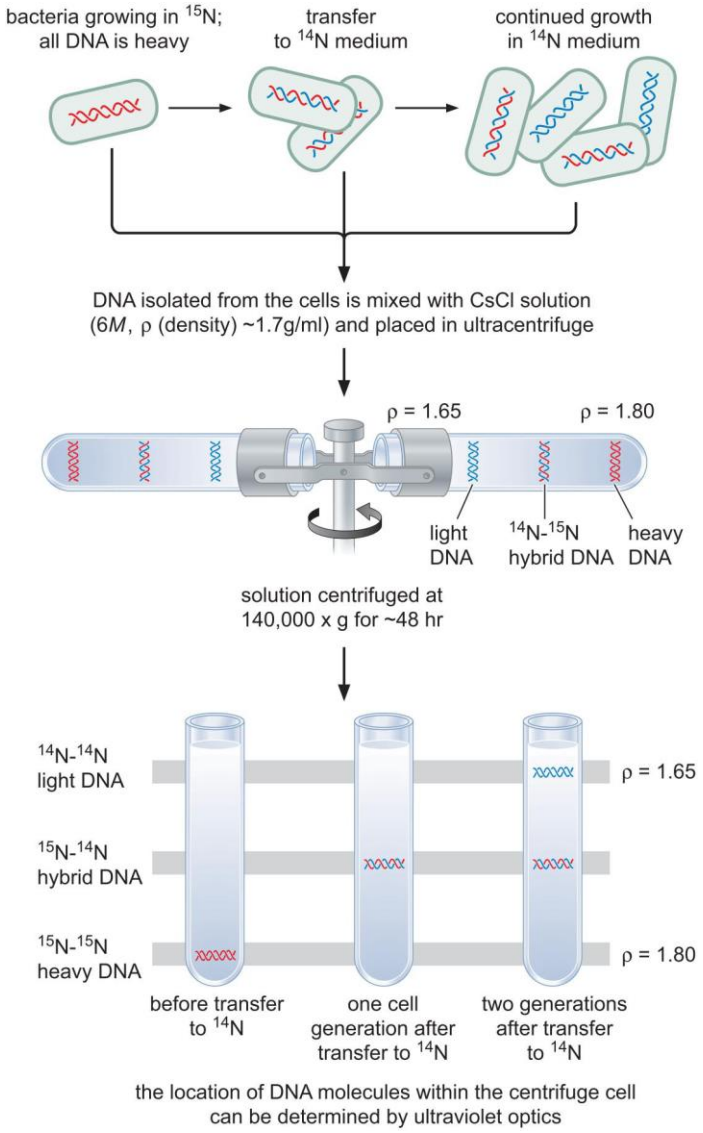
Radioisotopic labels have been employed in experiments bearing on the distribution of parental atoms among progeny molecules in several organisms.[6–9] We anticipated that a label which imparts to the DNA molecule an increased density might permit an analysis of this distribution by sedimentation techniques. To this end, a method was developed for the detection of small density differences among

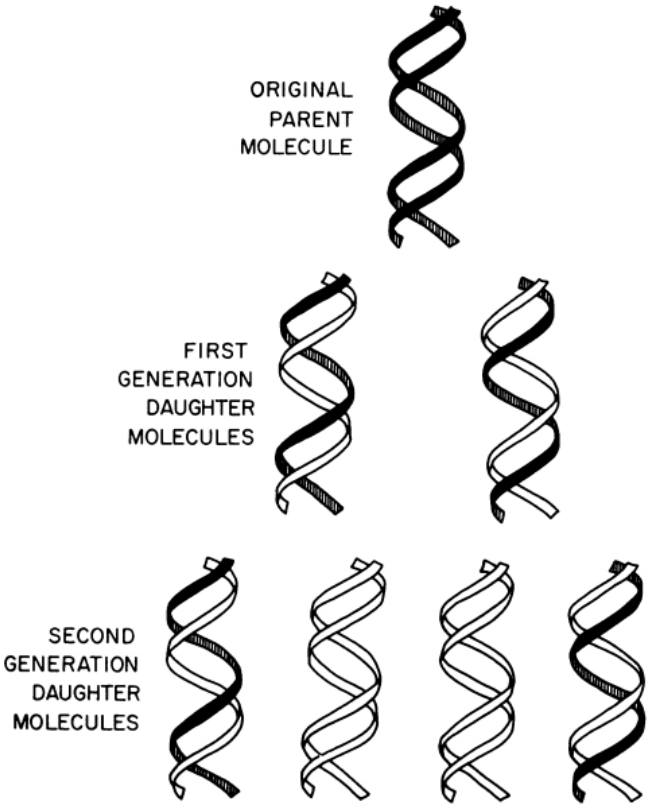


CsCl密度梯度离心可以富集相同分子量的DNA



N14，N15可以标记子代DNA分子

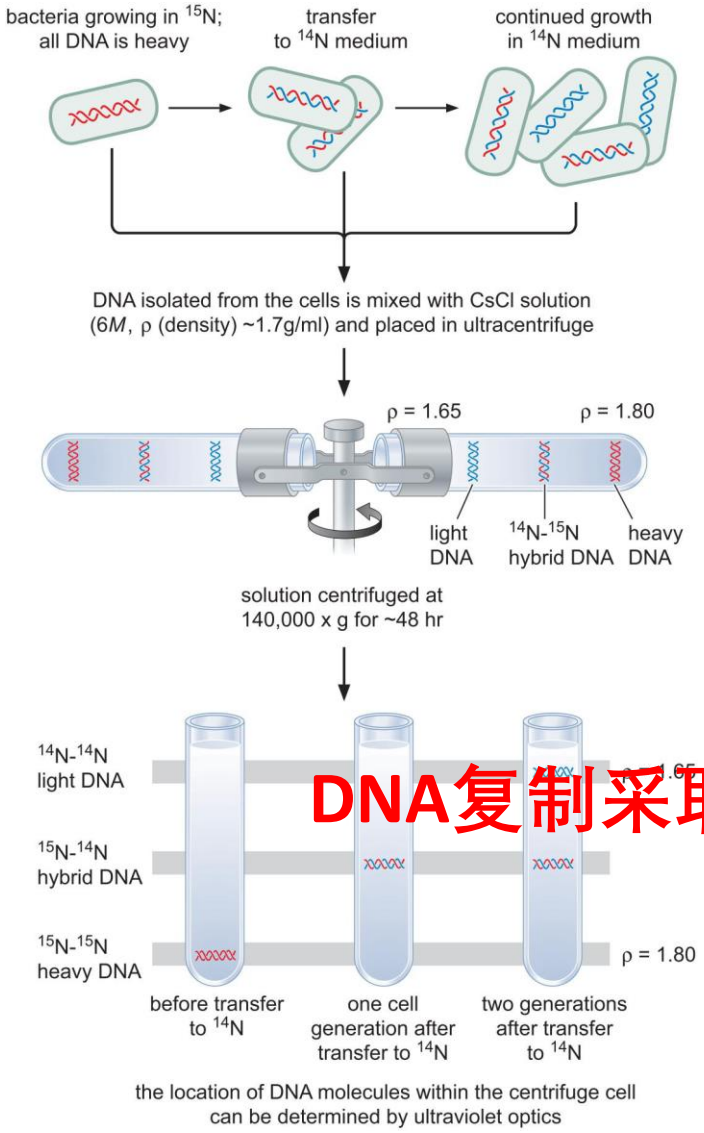


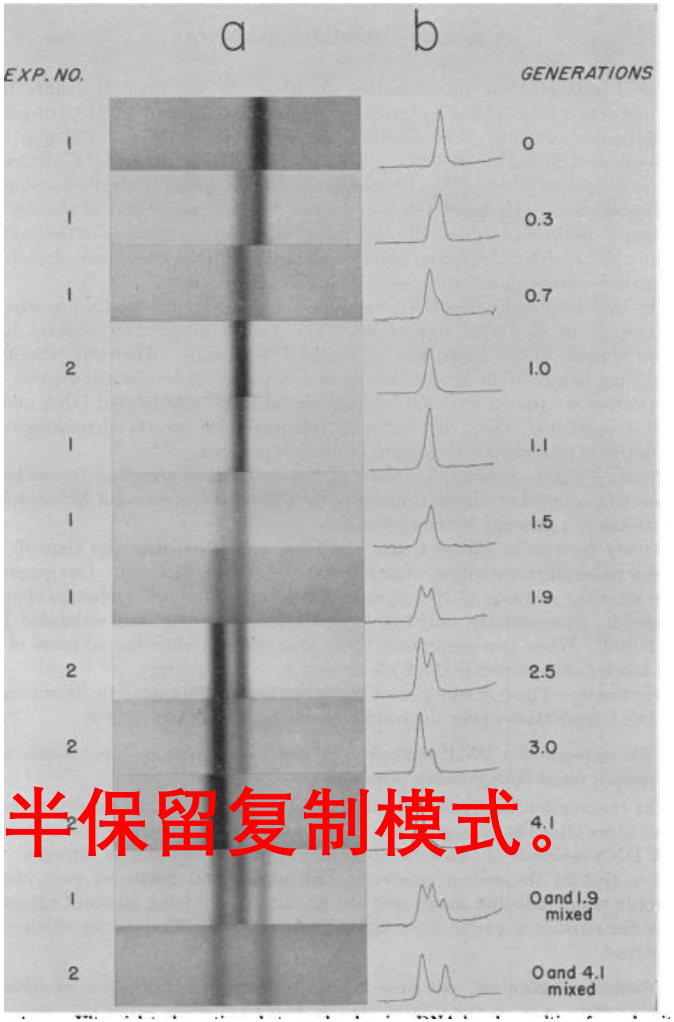


重链

杂合链

杂合链，轻重链





培养不同代数大肠杆菌的DNA密度梯度离心条带

DNA复制采取的是半保留复制模式。

第一节：DNA复制的一般特征

1.DNA复制采用哪种模式?

半保留复制模式

2. DNA复制是连续的，还是间断的?

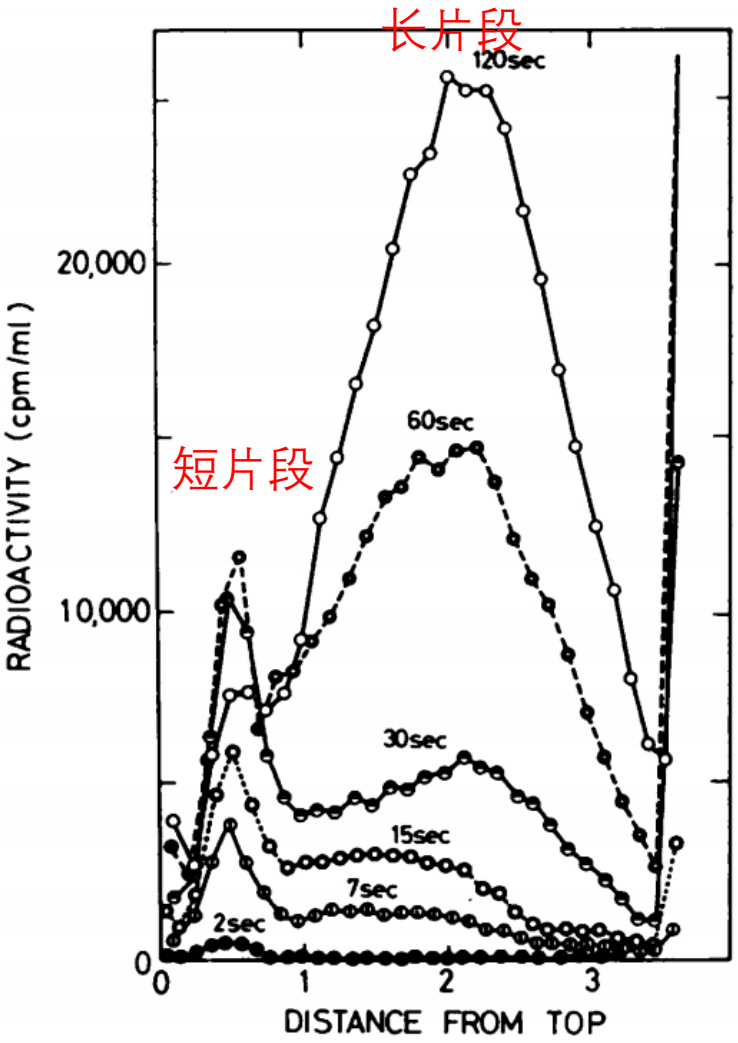
Reiji Okazaki

MECHANISM OF DNA CHAIN GROWTH, I. POSSIBLE DISCONTINUITY AND UNUSUAL SECONDARY STRUCTURE OF NEWLY SYNTHESIZED CHAINS

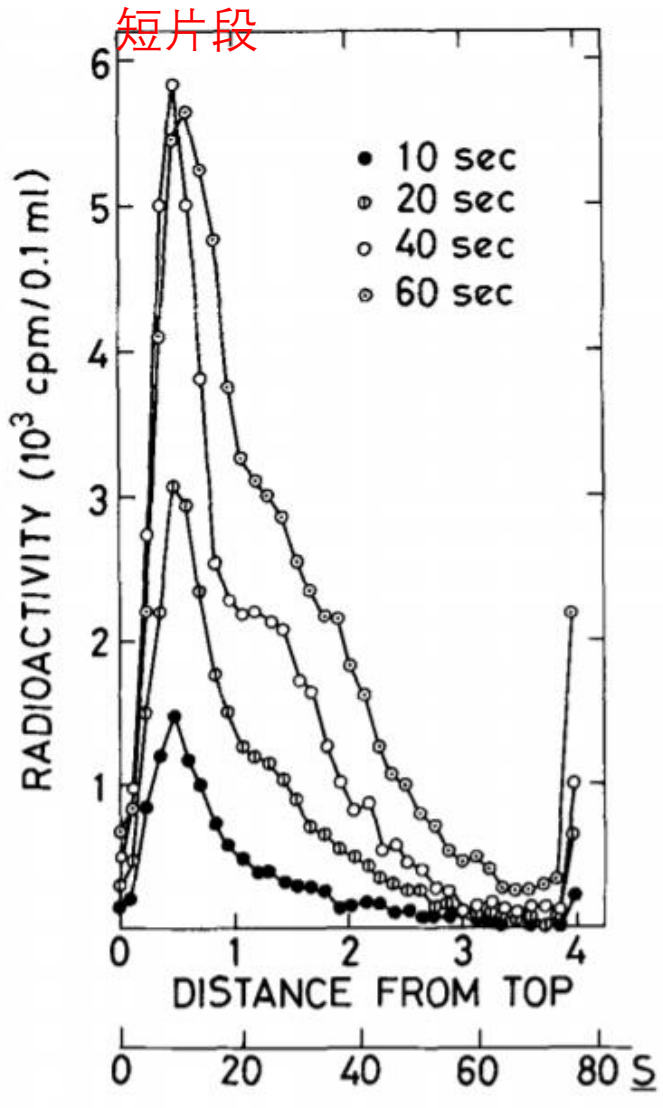
By Reiji Okazaki, Tuneko Okazaki, Kiwako Sakabe, Kazunori Sugimoto, and Akio Sugino

INSTITUTE OF MOLECULAR BIOLOGY AND DEPARTMENT OF CHEMISTRY, FACULTY OF SCIENCE, NAGOYA UNIVERSITY, NAGOYA, JAPAN

Communicated by Rollin D. Hotchkiss, December 14, 1967



T4 phage-infected cells



T4 *ts* B20-infected cells

Ligase mutant

Proc. Natl. Acad. Sci. USA
Vol. 75, No. 1, pp. 233–237, January 1978
Biochemistry

Uracil incorporation: A source of pulse-labeled DNA fragments in the replication of the *Escherichia coli* chromosome

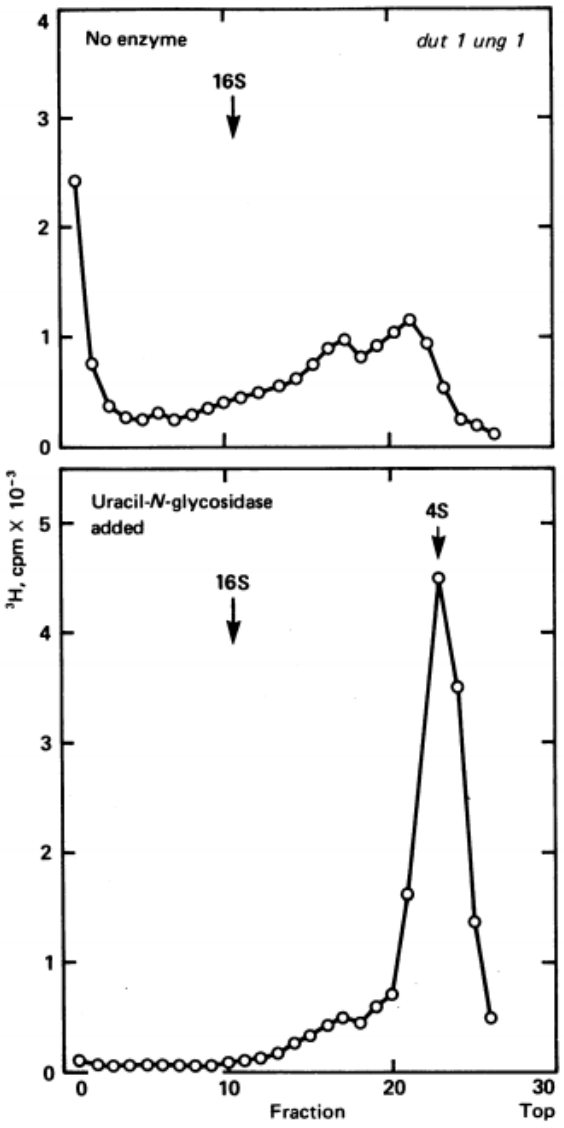
(Okazaki fragments/dUTPase/uracil *N*-glycosidase/discontinuous DNA replication)

BIK-KWOON TYE*†, JANICE CHIEN†, I. R. LEHMAN†, BRUCE K. DUNCAN‡, AND HUBER R. WARNER‡

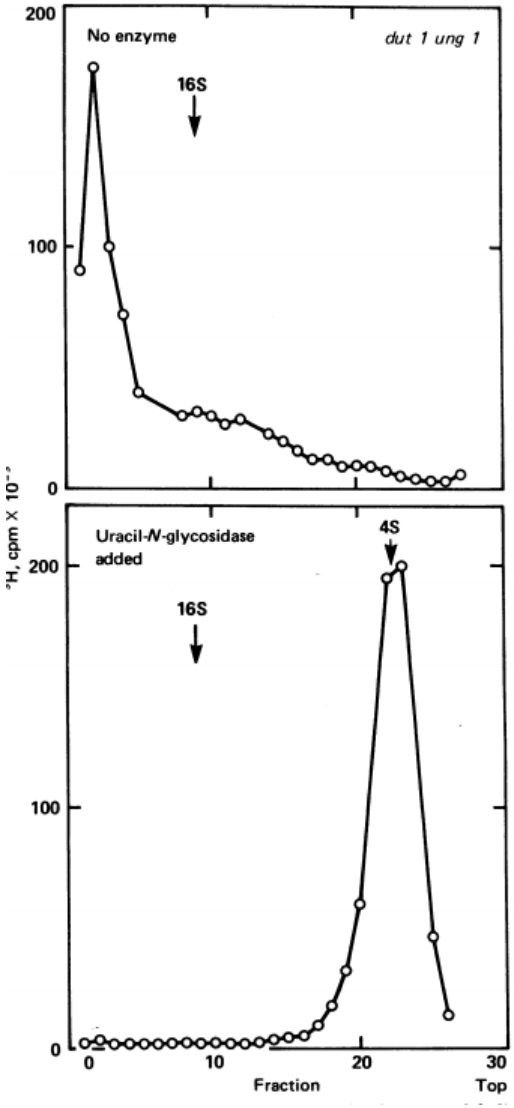
†Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305; and ‡Department of Biochemistry, College of Biological Sciences, University of Minnesota, St. Paul, Minnesota 55108

Contributed by Robert Lehman, November 3, 1977

dUTP的误用和校正修复是产生短片段DNA的原因



pulse-labeled with 3H-dTTP for 10 sec.



pulse-labeled with 3H-dTTP for 4 h.

Proc. Natl. Acad. Sci. USA
Vol. 75, No. 1, pp. 238–242, January 1978
Biochemistry

DNA intermediates at the *Escherichia coli* replication fork: Effect of dUTP

(cellophane disc system/asymmetric DNA synthesis/Okazaki pieces/uracil excision)

BALDOMERO M. OLIVERA

Department of Biology, University of Utah, Salt Lake City, Utah 84112

Communicated by Robert Lehman, November 3, 1977

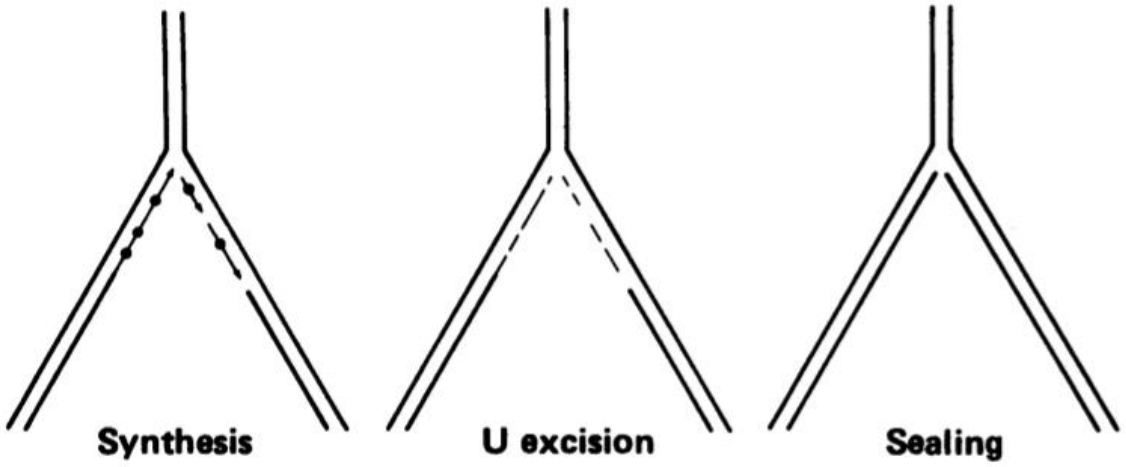


FIG. 6. A proposed model for the origin and sealing of Okazaki pieces. Dots in replication fork represent sites of uracil incorporation.

第一节：DNA复制的一般特征

1.DNA复制采用哪种模式?

半保留复制模式

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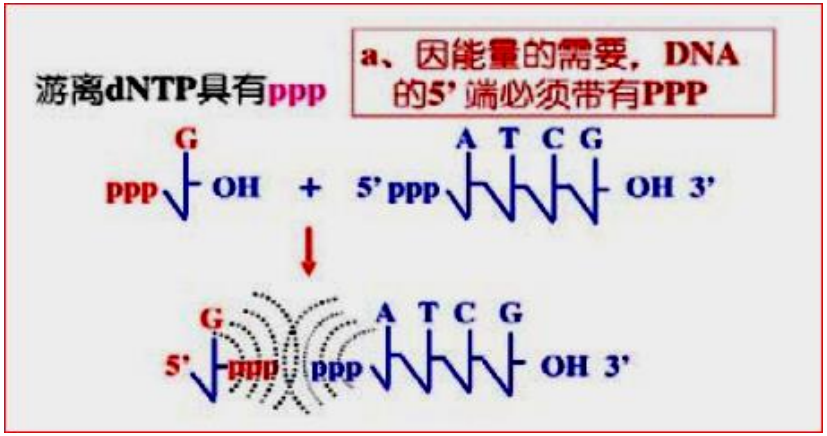
半不连续复制

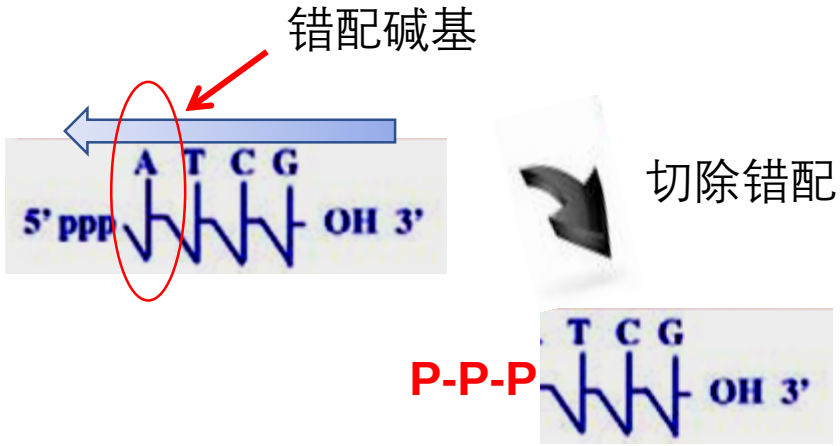
3. DNA新链的合成是否具有方向性?

DNA新链的合成的方向

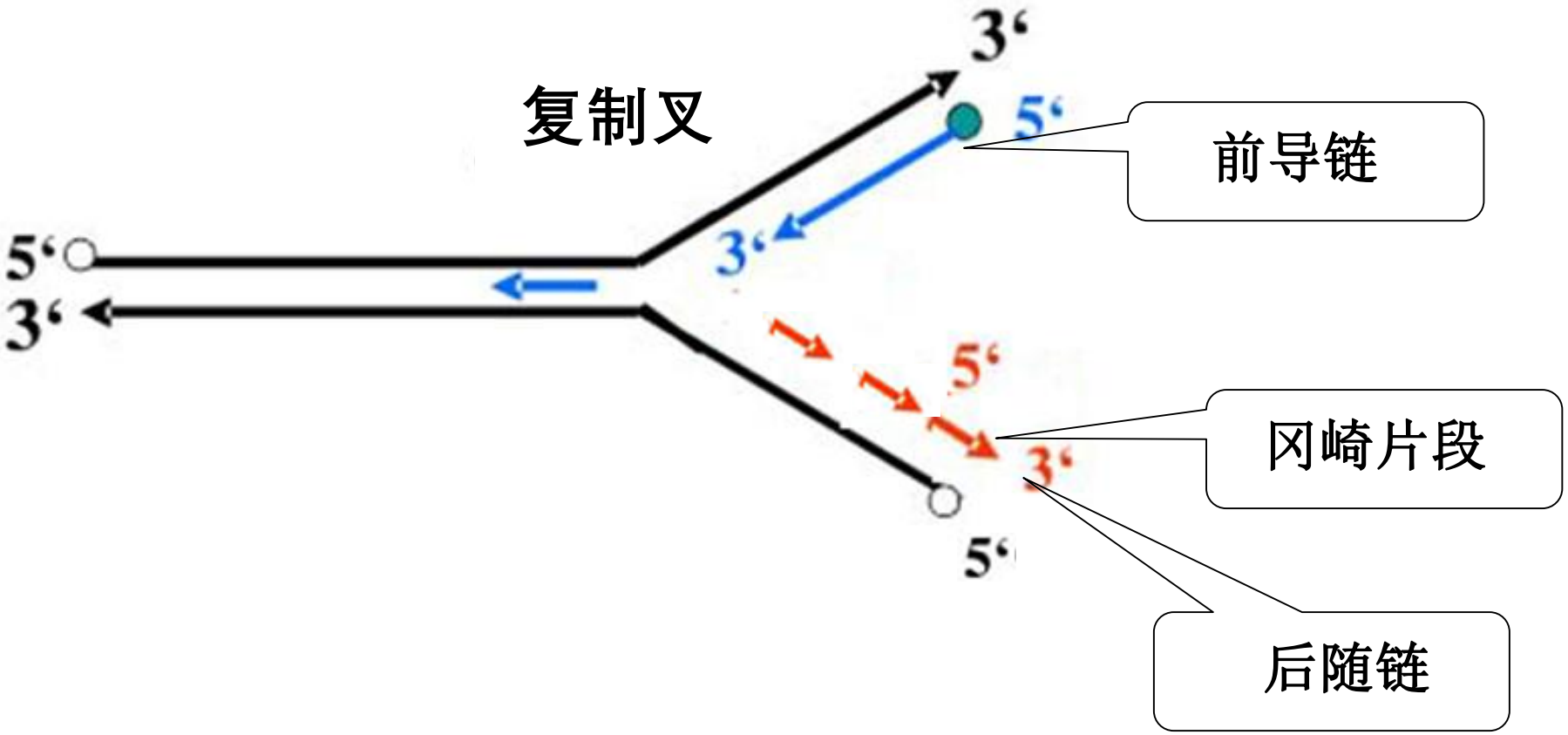
- DNA 聚合酶需要引物提供 3’—OH^-
- 若是3’→5’，则dNTP的5 ’—P-P-P与新生链上的5’—P-P-P存在静电斥力
- 若是3’→5’，则校正错配时，需要更多的酶系统和步骤。如下例：

DNA新链合成的方向：5’→3’





在5’端添加磷酸根以提供游离核苷酸的结合位点！


复制叉
3‘
5‘
前导链
5‘
3‘
3‘
5‘
5‘
冈崎片段
3‘
5‘
后随链

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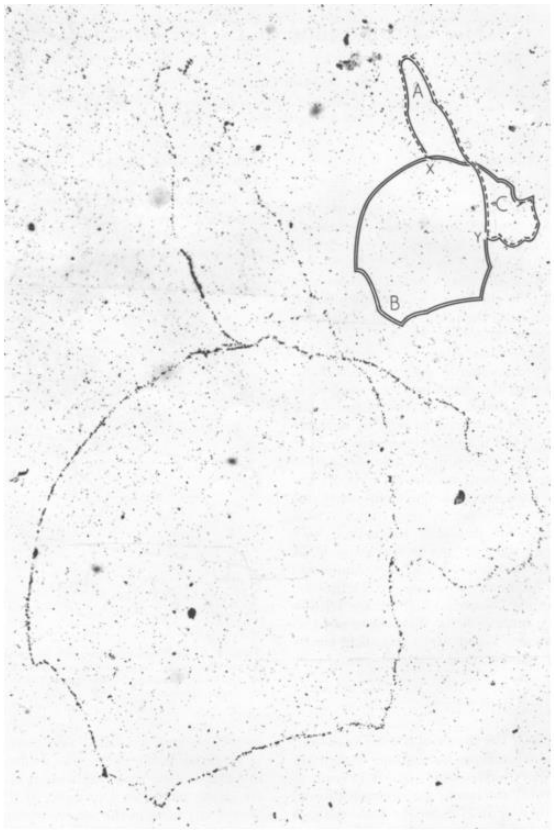
5’→3’

4. DNA复制的方向性?

The Chromosome of *Escherichia coli*

JOHN CAIRNS
Cold Spring Harbor Laboratory of Quantitative Biology,
Cold Spring Harbor, New York

H. John F. Cairns



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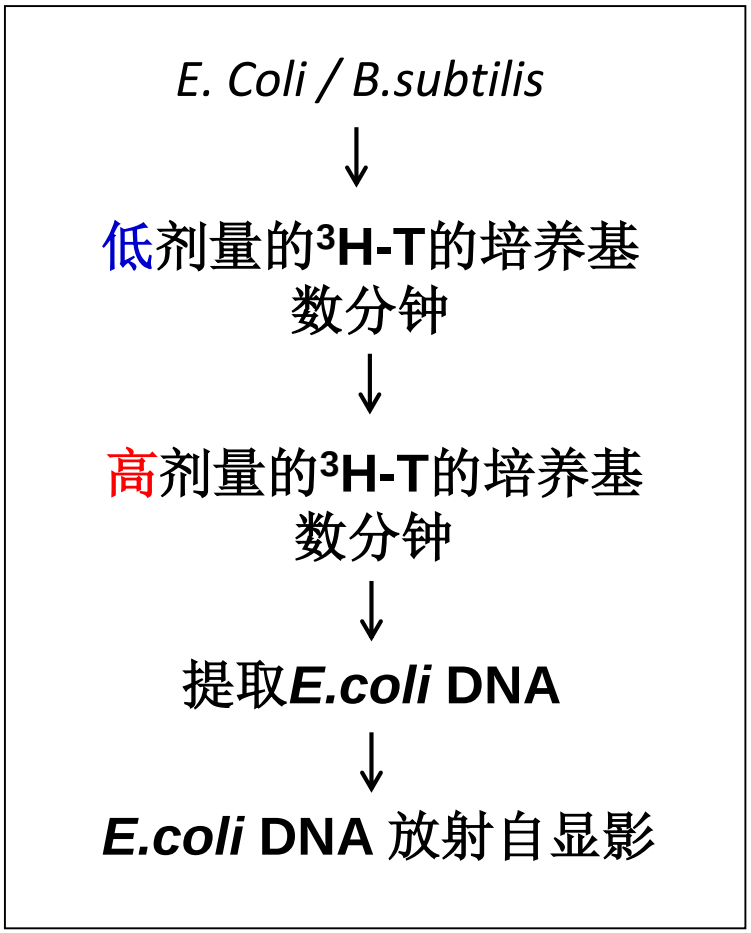
Autoradiograph of the chromosome of *E. coli* K12 after labeled with tritiated thymidine for two genenration

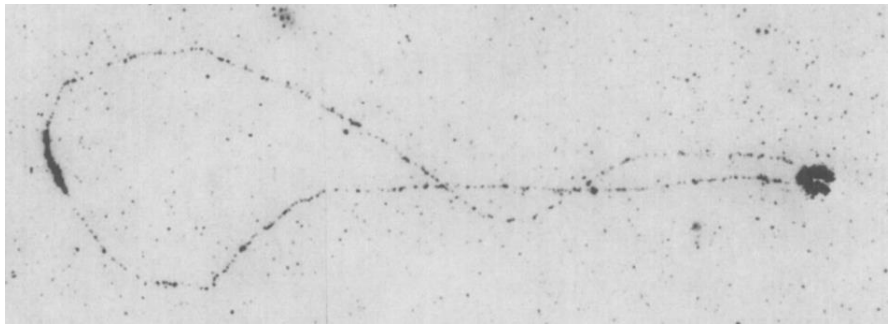

E. Coli / B.subtilis
低剂量的3H-T的培养基
数分钟
高剂量的3H-T的培养基
数分钟
提取E.coli DNA
E.coli DNA 放射自显影

Autoradiographic Evidence for Bidirectional DNA Replication in *Escherichia coli*

R. L. Rodriguez, M. S. Dalbey and C. I. Davern

Division of Natural Sciences
Kenneth V. Thimann Laboratories
University of California, Santa Cruz, Calif. 95060, U.S.A.

(*Received 26 September 1972*)

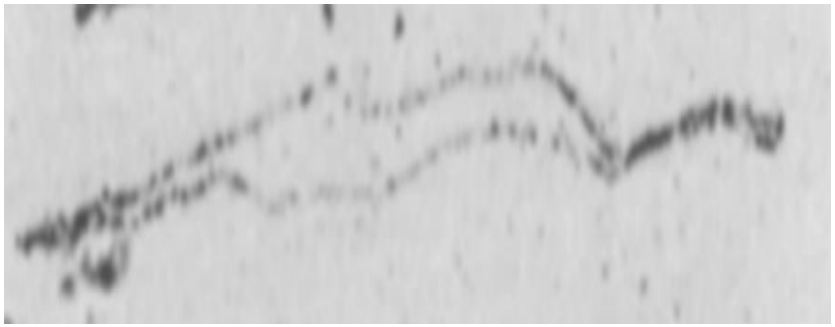


Bidirectional Chromosome Replication in *Bacillus subtilis*

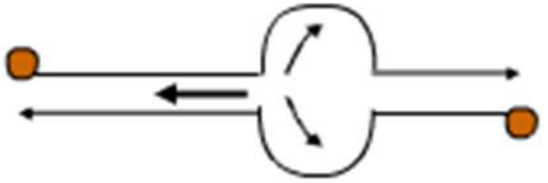
Elizabeth B. Gyurasits and R. G. Wake

Department of Biochemistry, University of Sydney
Sydney 2006, New South Wales, Australia

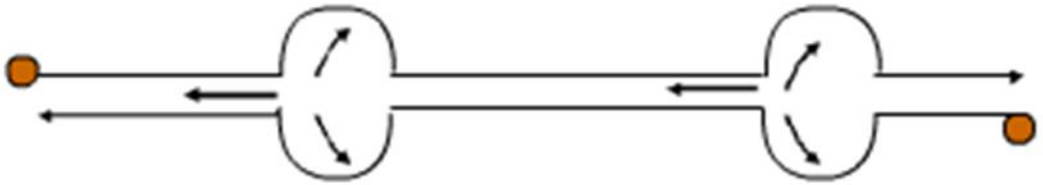
(*Received 5 July 1972*)

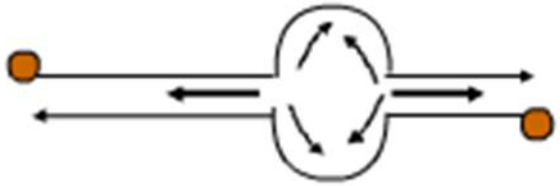


单起点、单方向

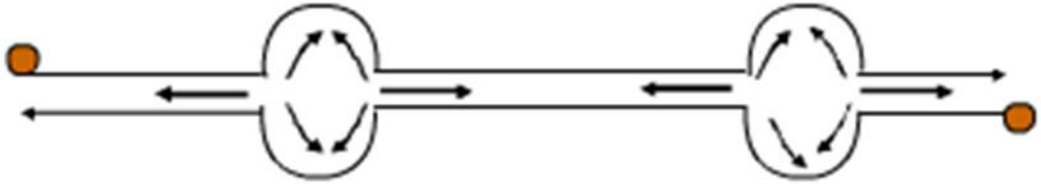


多起点、单方向





单起点、双方向



多起点、双方向

小结

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5’ →3’

4. DNA复制的方向性?

多样