Lecture 12:

DNA:
Replication Experiments

Readings (chapter 8)

Course 371

AIMS

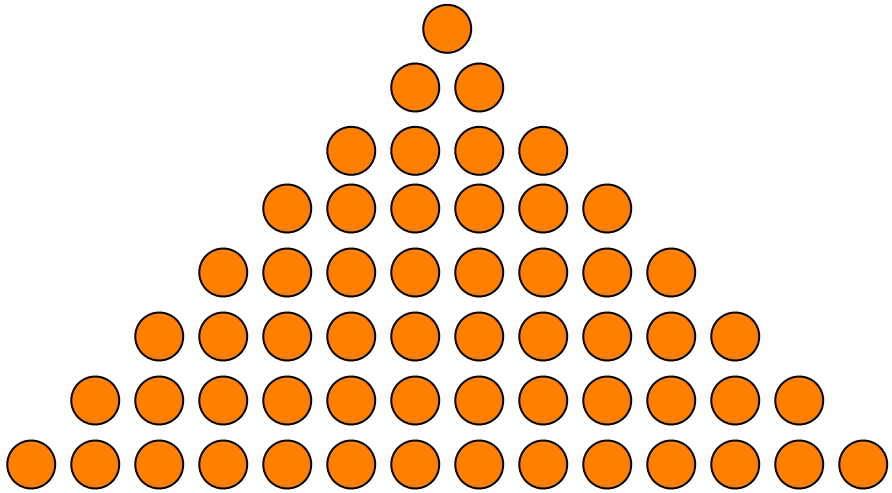
- Understand why DNA needs to be replicated.
- Understand the model of DNA replication and the experiment that led to the discovery.
- Understand Meselson and Stahl experiment.

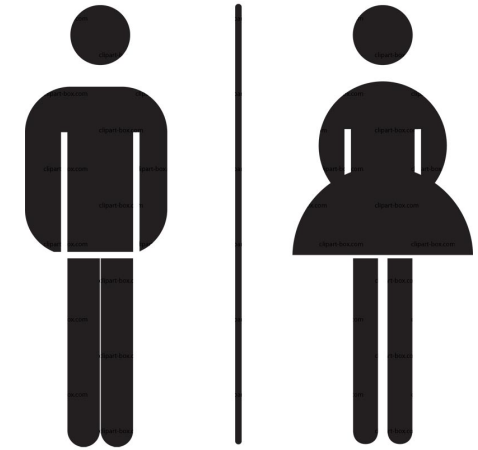
Why DNA replication?

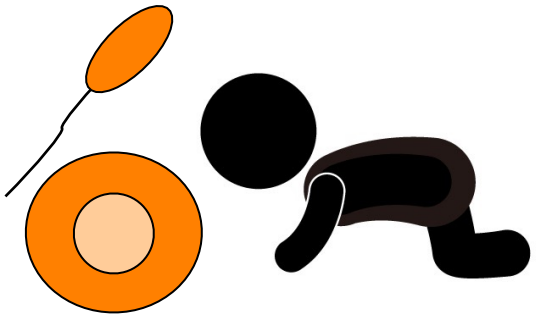
- DNA is the genetic code.
    - Pass genetic code from one generation of cells to the next (growth/ prokaryotes).
    - Pass genetic code from one generation of organisms to the next (multicellular).

Why DNA replication?

DNA replication is transmission and growth!







Why DNA replication?

What organisms transmission = growth?

DNA structure and replication

- The discovery of DNA structure hinted to a mechanism of DNA replication.

- Watson and Crick mentioned this in their publication.

- **What parts of the DNA structure give hints?**
    - Double strands.
    - Complementary basepairing.

DNA structure and replication

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three inter-twined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate di-ester groups joining β-D-deoxy-ribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

This figure is purely diagrammatic. The two ribbons symbolize the two phosphate–sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxy-ribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereo-

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on inter-atomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at King's College, London. One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

J. D. WATSON
F. H. C. CRICK

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems,
Cavendish Laboratory, Cambridge.
April 2.

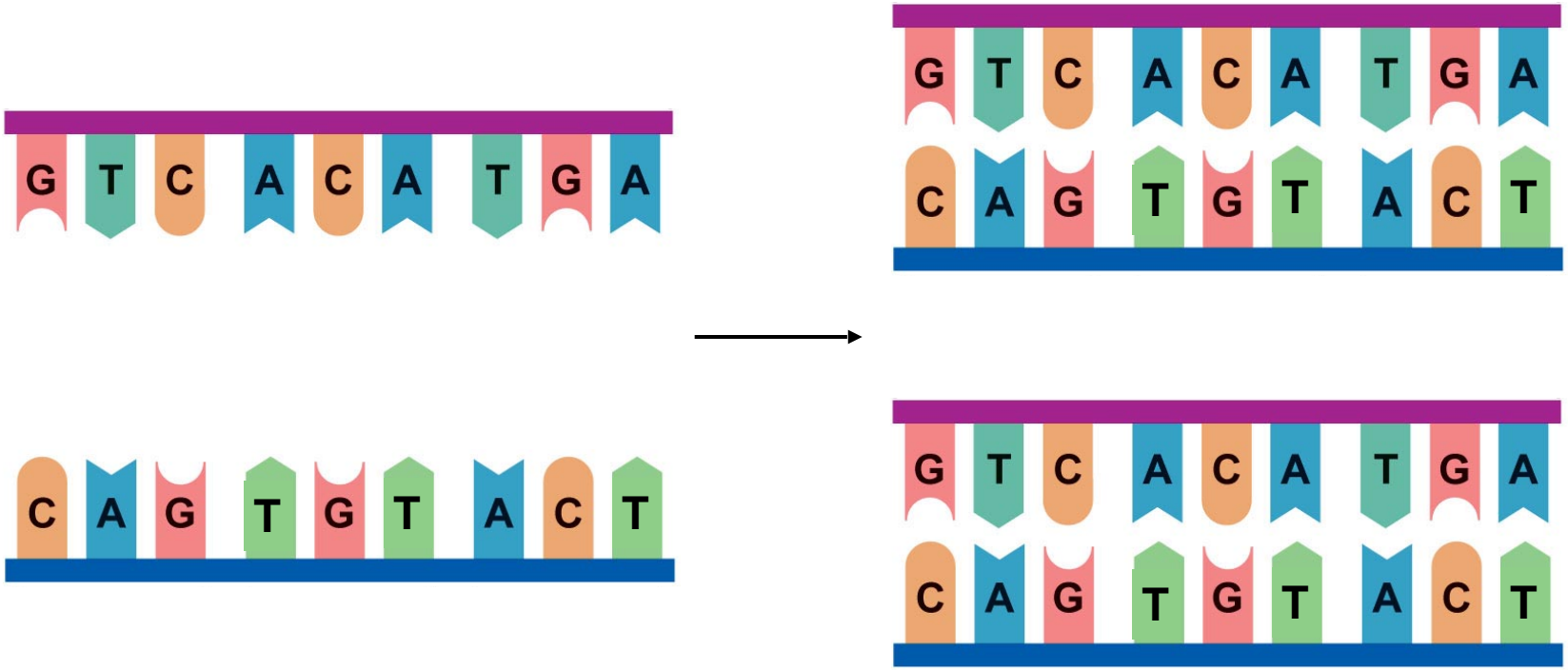
[1] Pauling, L., and Corey, R. B., *Nature*, **171**, 346 (1953); *Proc. U.S. Nat. Acad. Sci.*, **39**, 84 (1953).
[2] Furberg, S., *Acta Chem. Scand.*, **6**, 634 (1952).
[3] Chargaff, E., for references see Zamenhof, S., Brawerman, G., and Chargaff, E., *Biochim. et Biophys. Acta*, **9**, 402 (1952).
[4] Wyatt, G. R., *J. Gen. Physiol.*, **36**, 201 (1952).
[5] Astbury, W. T., Symp. Soc. Exp. Biol. 1, Nucleic Acid, 66 (Camb. Univ. Press, 1947).
[6] Wilkins, M. H. F., and Randall, J. T., *Biochim. et Biophys. Acta*, **10**, 192 (1953).

Each strand can serve as the template to be copied

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Do you remember DNA structure?

If we separate the strands, we can you predict the complementary sequence!



Each strand can serve as the template to be copied

Replication

• We will go over DNA replication in prokaryotes and eukaryotes covering:

- Process.
- Enzymes involved.

BUT

Before that when need to know what is the model of DNA replication

How is DNA replicated?

Three hypotheses:

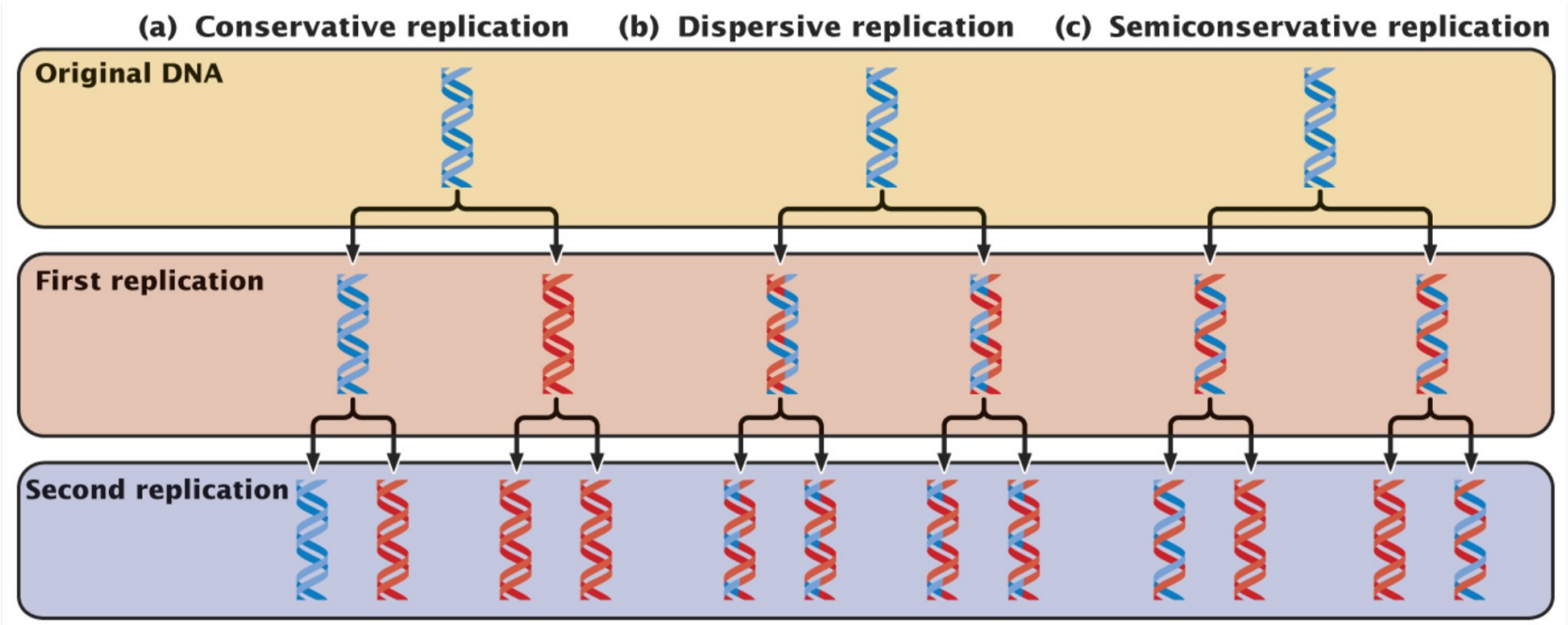
1. Conservative replication: replicated DNA results in one old double strand DNA and one new double strands of DNA.

2. Dispersive replication: replicated DNA results in each double strands with segments of old and new DNA.

3. Semi-conservative replication: each double strand has one old strand and one new strand.

How is DNA replicated?

- **Three hypotheses:**
  - Conservative replication
  - Dispersive replication
  - Semi-conservative replication



How is DNA replicated?

Watson and Crick proposed a mechanism for DNA replication **BUT** needed evidence.

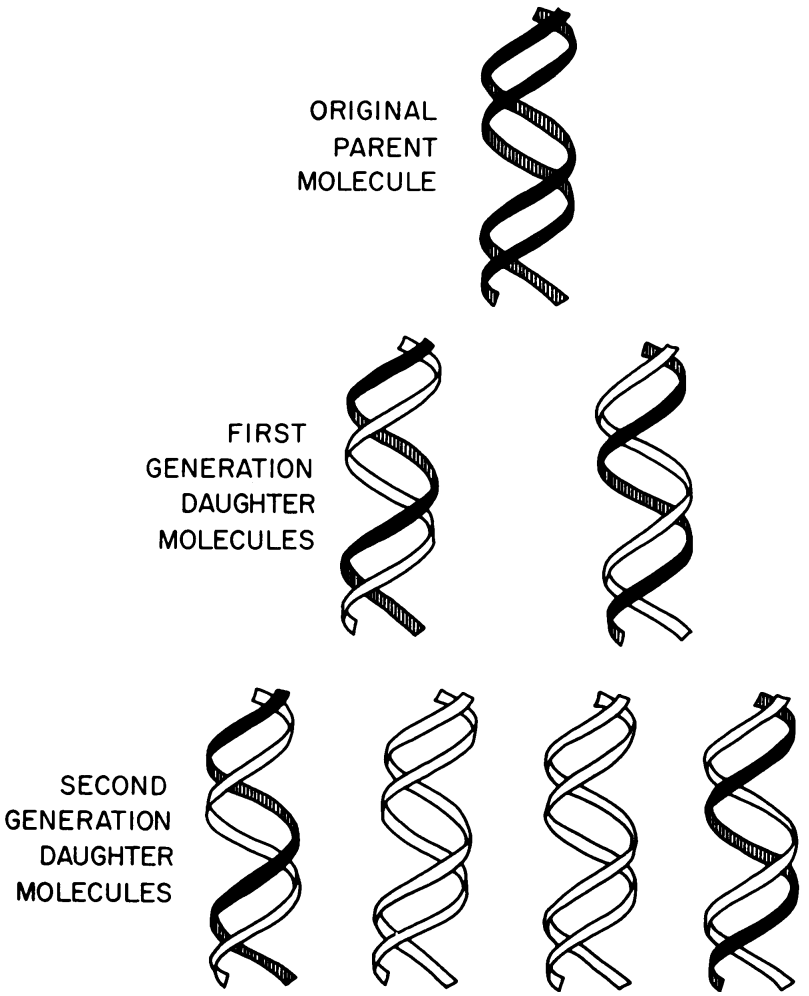


FIG. 6.—Illustration of the mechanism of DNA duplication proposed by Watson and Crick. Each daughter molecule contains one of the parental chains (*black*) paired with one new chain (*white*). Upon continued duplication, the two original parent chains remain intact, so that there will always be found two molecules each with one parental chain.

Meselson M. and Stahl FW. (1958) THE REPLICATION OF DNA IN ESCHERICHIA COLI. Proc Natl Acad Sci USA. 44(7):671-82.

Meselson and Stahl experiment

- Matt Meselson and Frank Stahl designed **the most beautiful experiment in biology.**

- The experiment tests the DNA replication models.

- They used bacteria grown in a media of a heavy isotope of nitrogen .

Meselson and Stahl experiment

*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

Meselson M. and Stahl FW. (1958) THE REPLICATION OF DNA IN ESCHERICHIA COLI. Proc Natl Acad Sci USA. 44(7):671-82.

Meselson and Stahl experiment

Do you know heavy isotopes?

Meselson and Stahl experiment

- In nature, some elements have different isotopes which they differ in the number of neutrons.
- The difference in the number of neutrons makes the isotopes' atoms heavier.

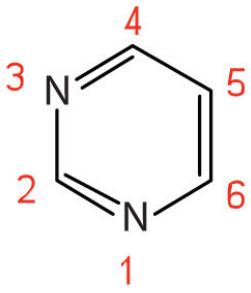
Meselson and Stahl experiment

- The abundant nitrogen is N^{14} where there are 7 protons and 7 neutrons and it weights 14g/ mole.
- N^{15} is a stable isotope of nitrogen and has 7 protons and 8 neutrons and weights 15g/mole.

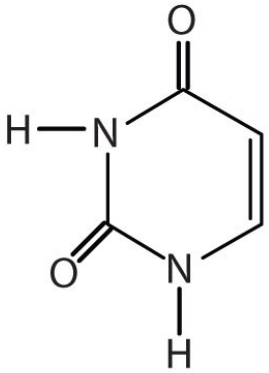
Meselson and Stahl experiment

Why heavy nitrogen isotope N^{15}?

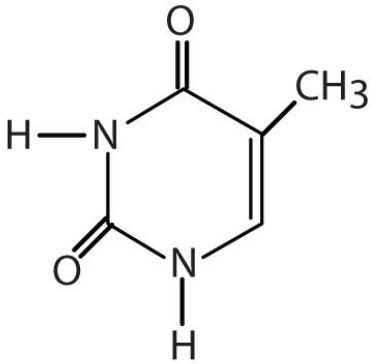
Meselson and Stahl experiment



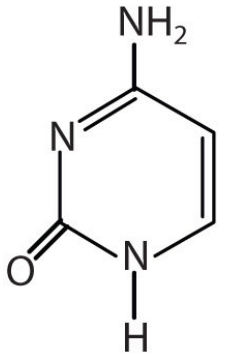
Pyrimidine



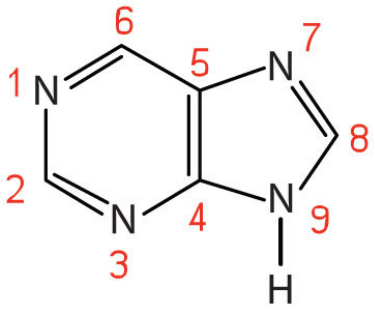
Uracil (U)
RNA only



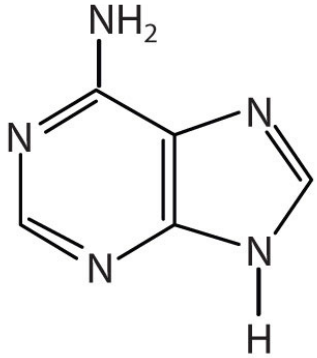
Thymine (T)
DNA only



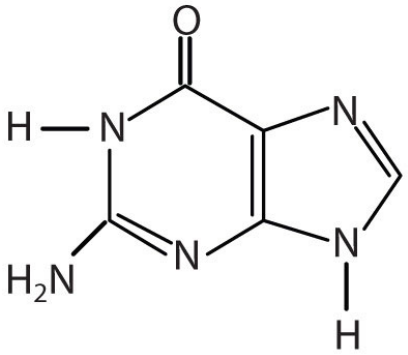
Cytosine (C)
both DNA and RNA



Purine



Adenine (A)

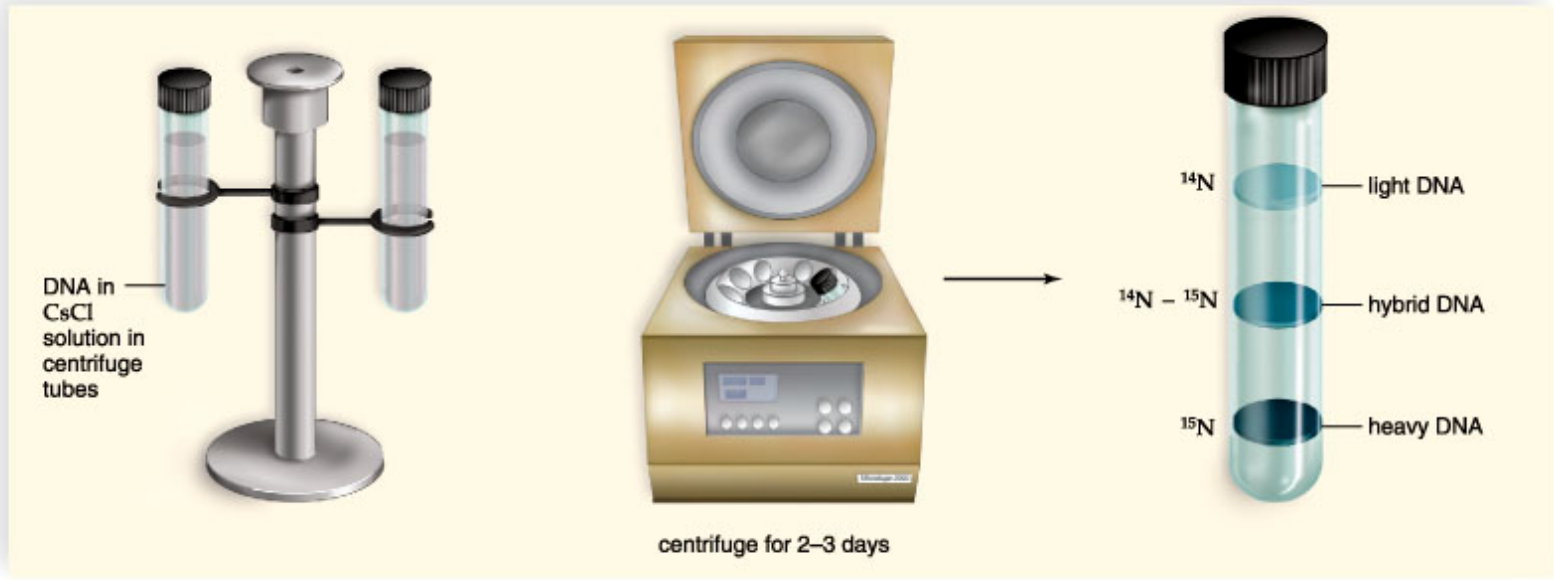


Guanine (G)

Remember DNA bases contain **Nitrogen**

Meselson and Stahl experiment

The method of visualizing and detecting N^{15} vs. N^{14} DNA was **density gradient centrifugation**



a. Possible results when DNA is centrifuged in CsCl

Meselson and Stahl experiment

Density of DNA with N^{14} and N^{15} can be detected

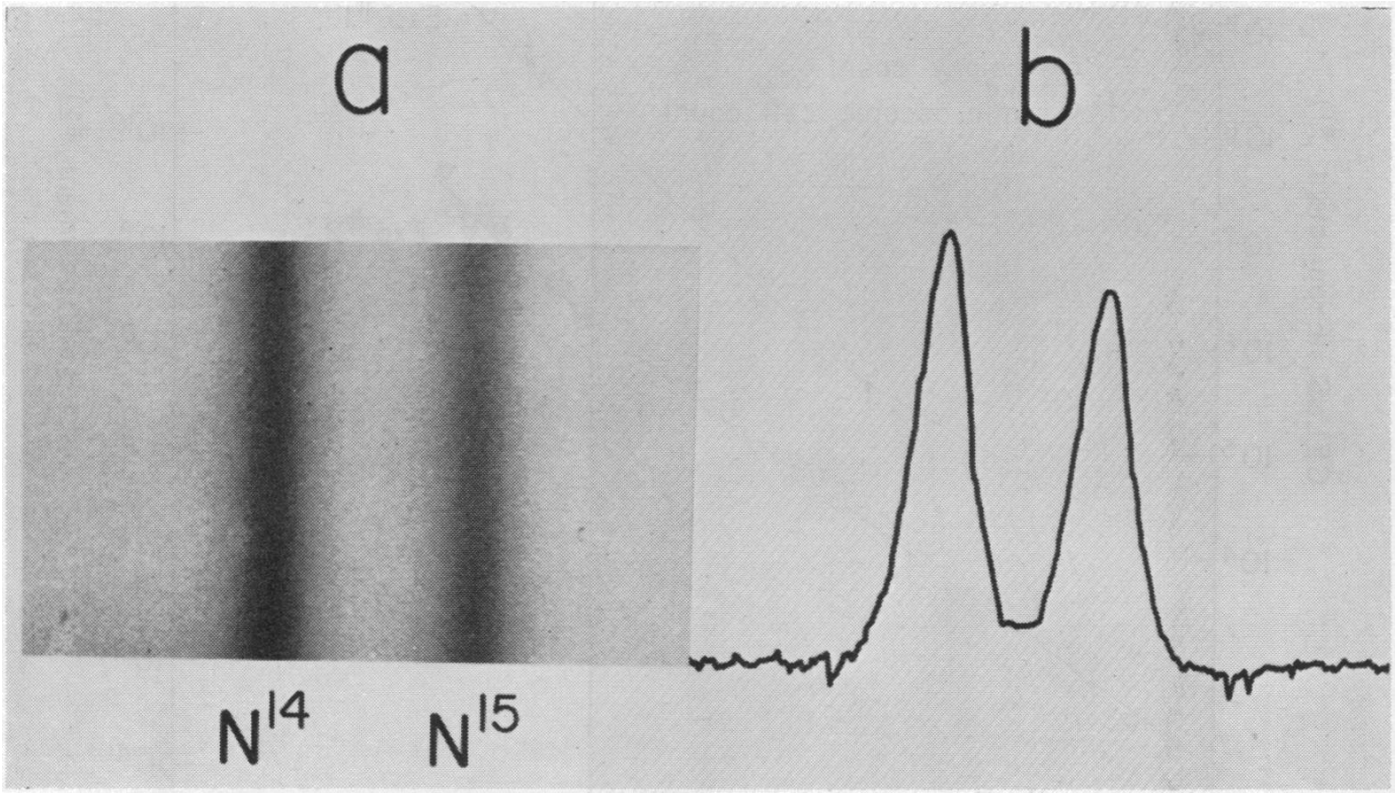


FIG. 2—*a:* The resolution of N^{14} DNA from N^{15} DNA by density-gradient centrifugation. A mixture of N^{14} and N^{15} bacterial lysates, each containing about 10^8 lysed cells, was centrifuged in CsCl solution as described in the text. The photograph was taken after 24 hours of centrifugation at 44,770 rpm. *b:* A microdensitometer tracing showing the DNA distribution in the region of the two bands of Fig. 2*a*. The separation between the peaks corresponds to a difference in buoyant density of 0.014 gm. $cm.^{-3}$

Meselson M. and Stahl FW. (1958) THE REPLICATION OF DNA IN ESCHERICHIA COLI. Proc Natl Acad Sci USA. 44(7):671-82.

Meselson and Stahl experiment

The experiment:

1) Grow bacteria in a media containing the heavy N^{15} for many generations.

WHY?

Result: all bacteria has N^{15} in their DNA.

Meselson and Stahl experiment

The experiment:

2) Transfer some N^{15} bacteria to grow in N^{14} media and allow to grow for several generations.

WHY?

Result: DNA of newly divided bacteria will have N^{14} instead of N^{15}.

Meselson and Stahl experiment

Why (negative) time?

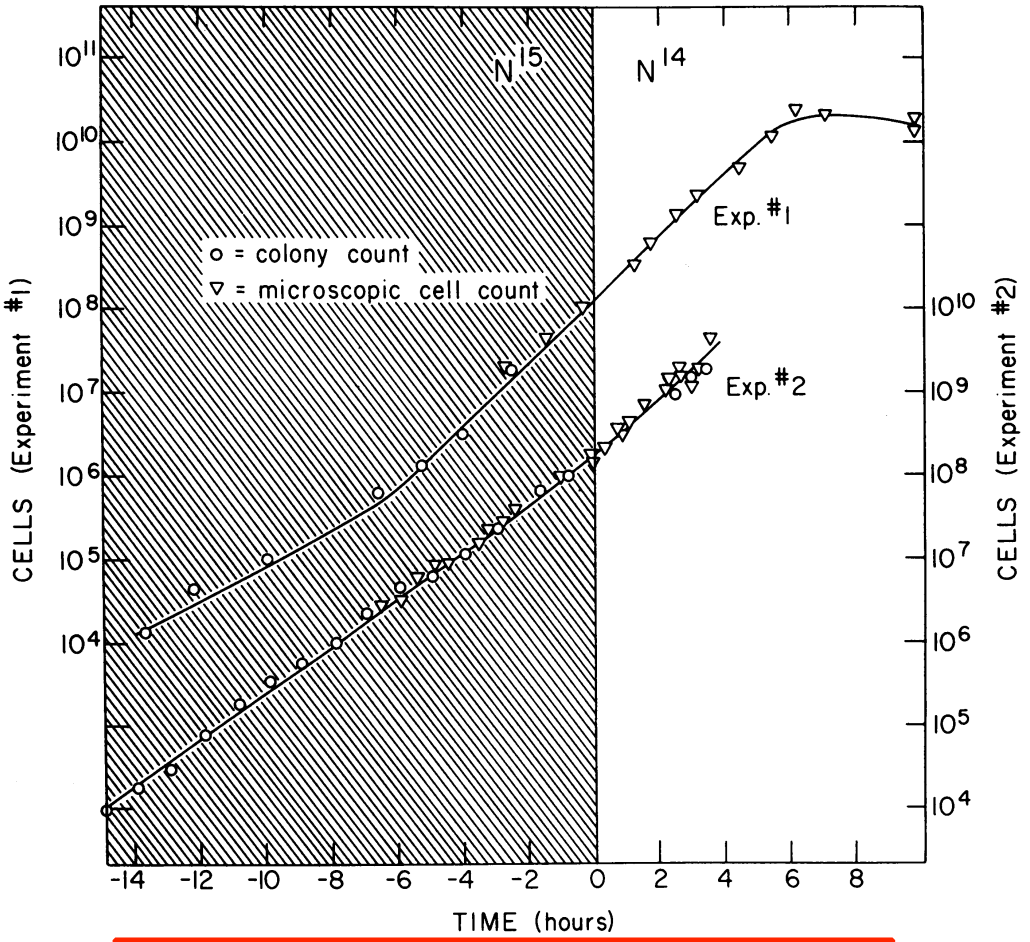


FIG. 3.—Growth of bacterial populations first in N^{15} and then in N^{14} medium. The values on the ordinates give the actual titers of the cultures up to the time of addition of N^{14}. Thereafter, during the period when samples were being withdrawn for density-gradient centrifugation, the actual titer was kept between 1 and 2 × 10^8 by additions of fresh medium. The values on the ordinates during this later period have been corrected for the withdrawals and additions. During the period of sampling for density-gradient centrifugation, the generation time was 0.81 hours in Experiment 1 and 0.85 hours in Experiment 2.

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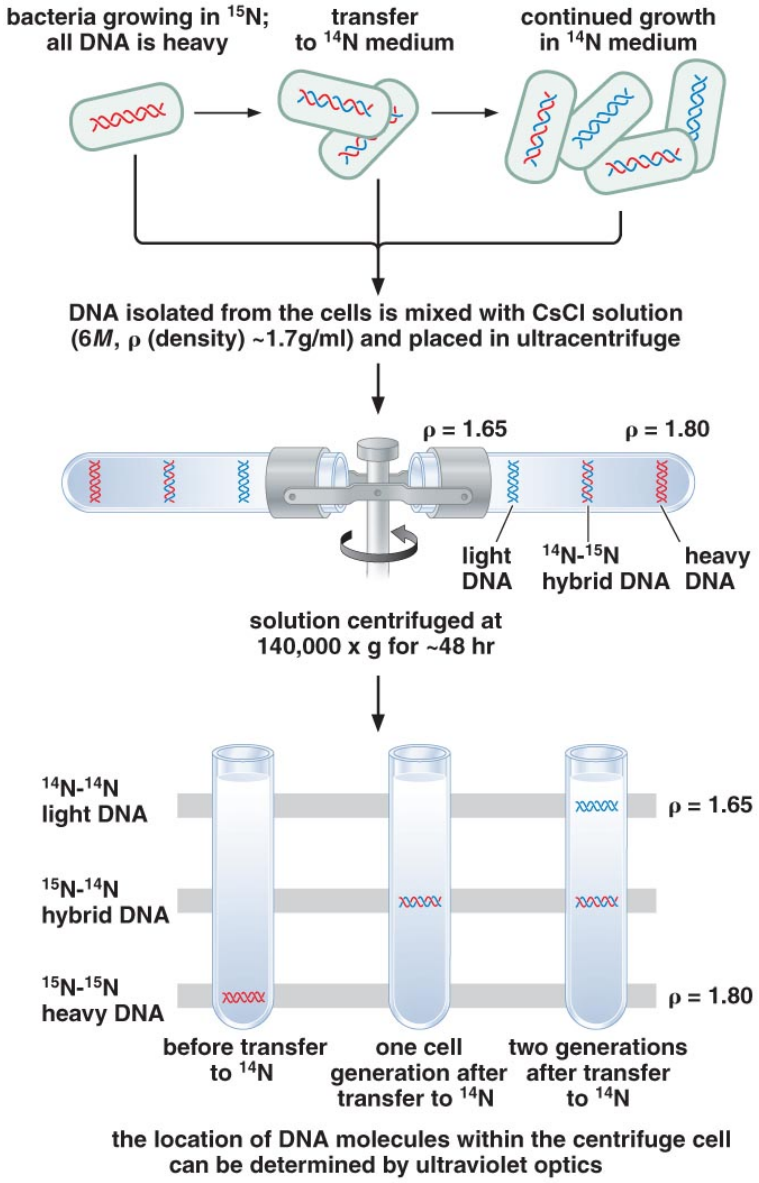
Meselson and Stahl experiment

The experiment:

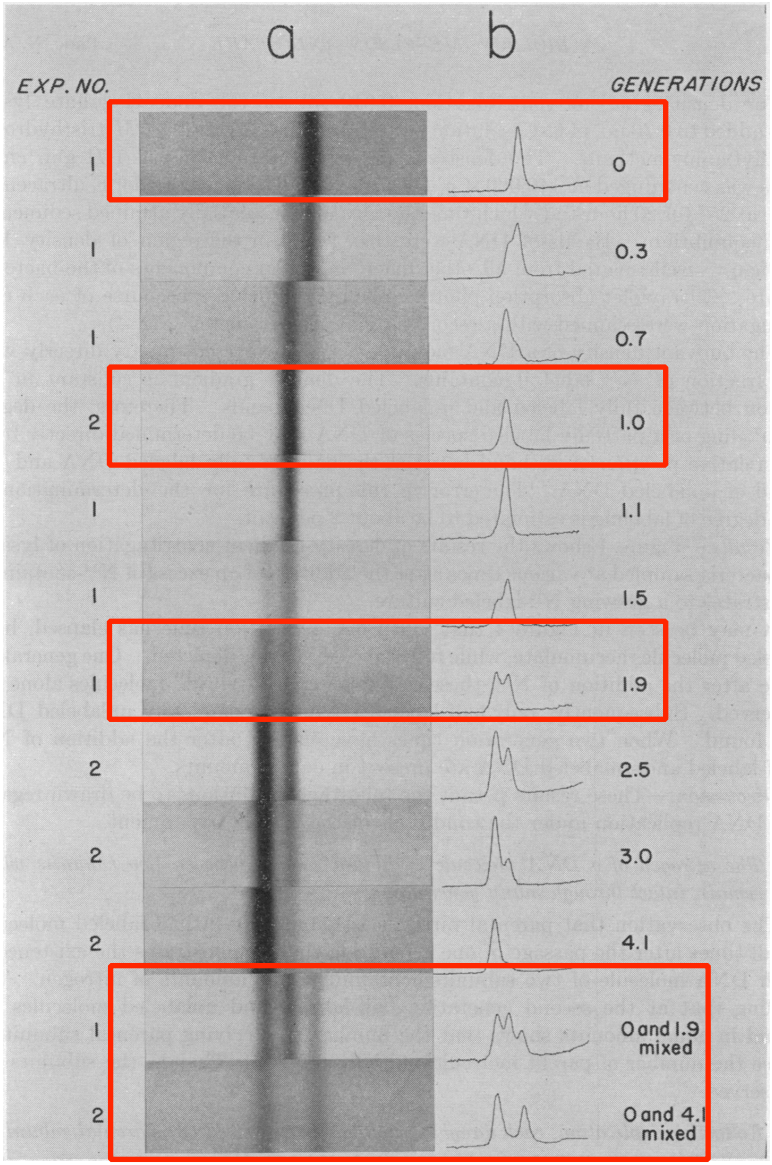
3) Take samples from the growing bacteria at different time and study the density of the DNA of the cells.

Meselson and Stahl experiment



Simply the idea is to mark the old DNA and see what happens to the newly synthesized DNA.

Meselson and Stahl experiment



- At generation (0), how many DNA species are in the sample?
- How many bands?
- Identity of DNA species?
- What about other G=1, G=2?
- Any controls?

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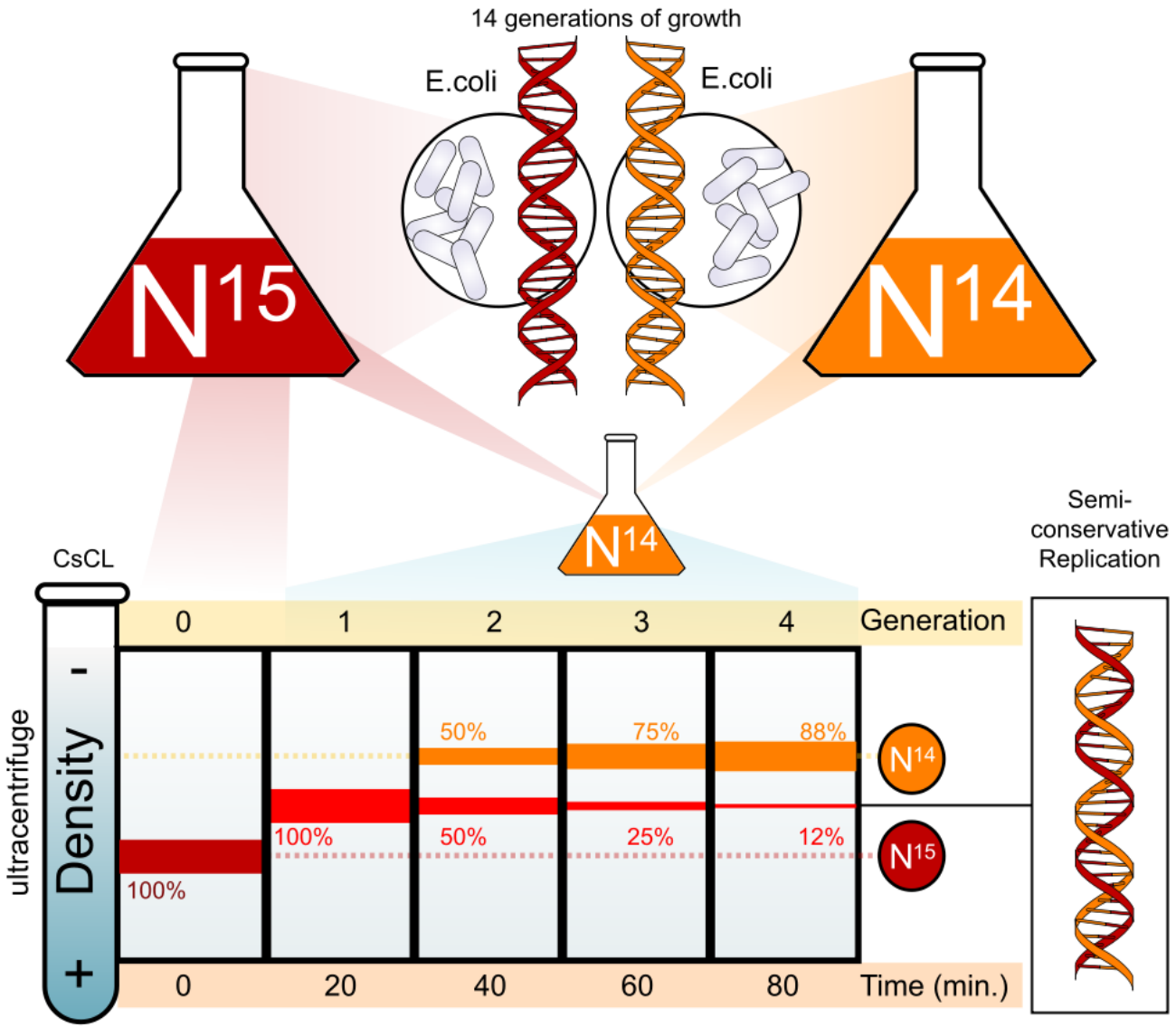
Meselson and Stahl experiment

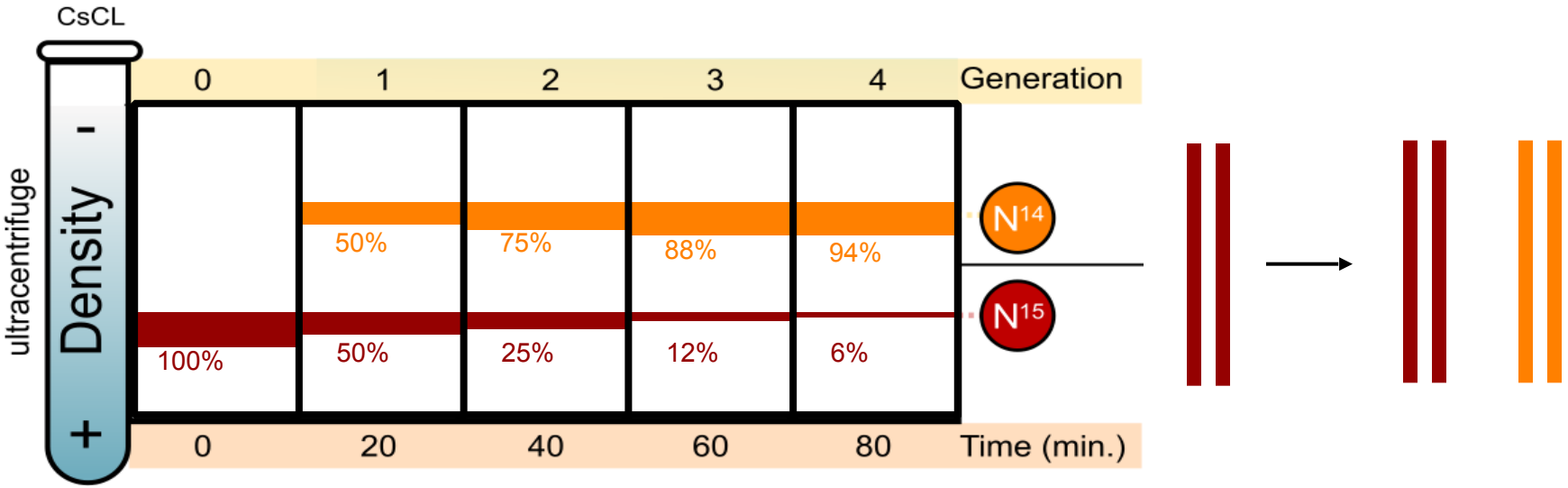
Let's look in details at the experiment and the expected results of each hypothesis

Meselson and Stahl experiment



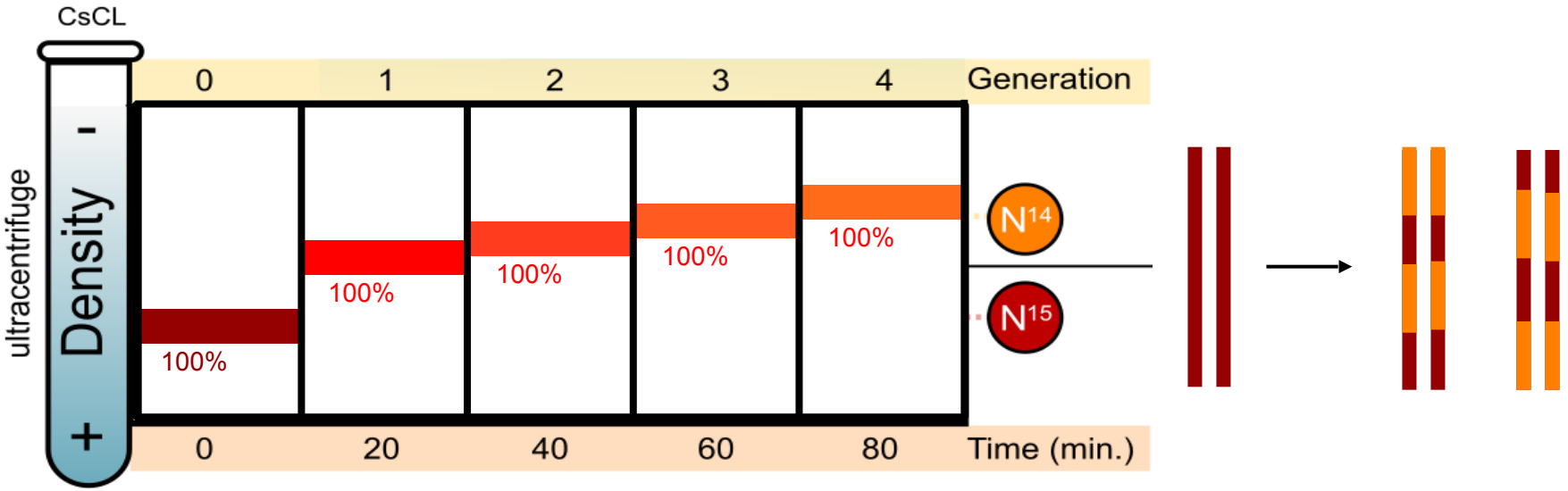
What would the results be if another model?

Conservative replication



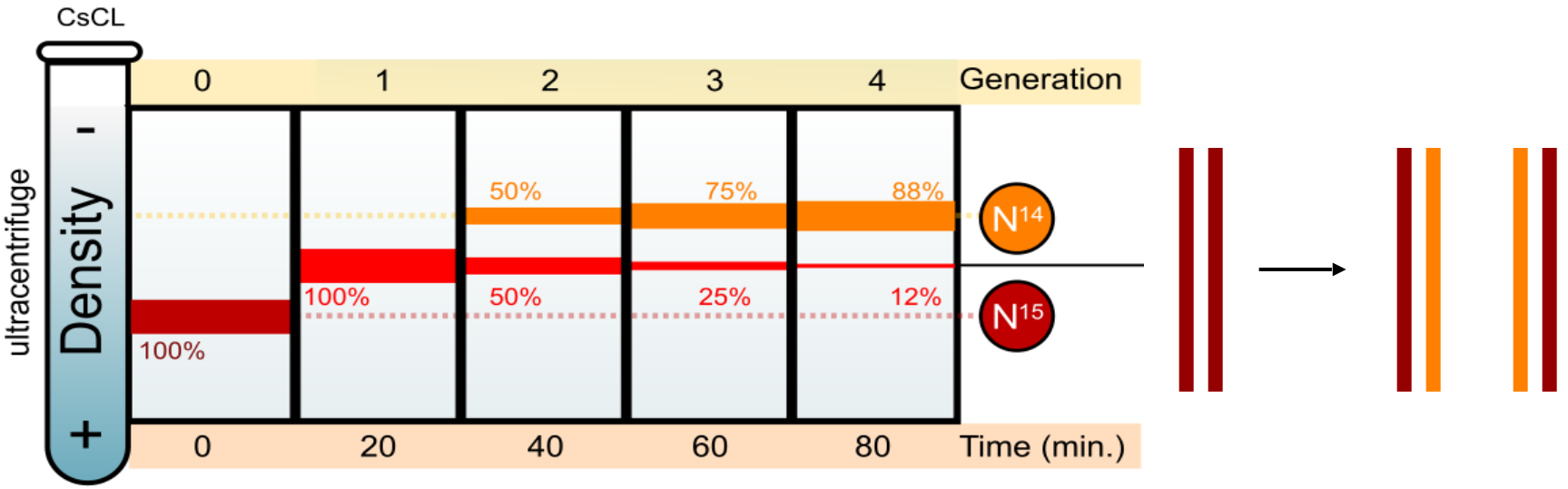
What would the results be if another model?

Dispersive replication



What would the results be if another model?

Semi-conservative replication



Conclusions

1. *The nitrogen of a DNA molecule is divided equally between two subunits which remain intact through many generations.*

The observation that parental nitrogen is found only in half-labeled molecules at all times after the passage of one generation time demonstrates the existence in each DNA molecule of two subunits containing equal amounts of nitrogen. The finding that at the second generation half-labeled and unlabeled molecules are found in equal amounts shows that the number of surviving parental subunits is twice the number of parent molecules initially present. That is, the subunits are conserved.

2. *Following replication, each daughter molecule has received one parental subunit.*

The finding that all DNA molecules are half-labeled one generation time after the addition of N^{14} shows that each daughter molecule receives one parental subunit.[14] If the parental subunits had segregated in any other way among the daughter molecules, there would have been found at the first generation some fully labeled and some unlabeled DNA molecules, representing those daughters which received two or no parental subunits, respectively.

3. *The replicative act results in a molecular doubling.*

This statement is a corollary of conclusions 1 and 2 above, according to which each parent molecule passes on two subunits to progeny molecules and each progeny molecule receives just one parental subunit. It follows that each single molecular reproductive act results in a doubling of the number of molecules entering into that act.

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The semi-conservative replication

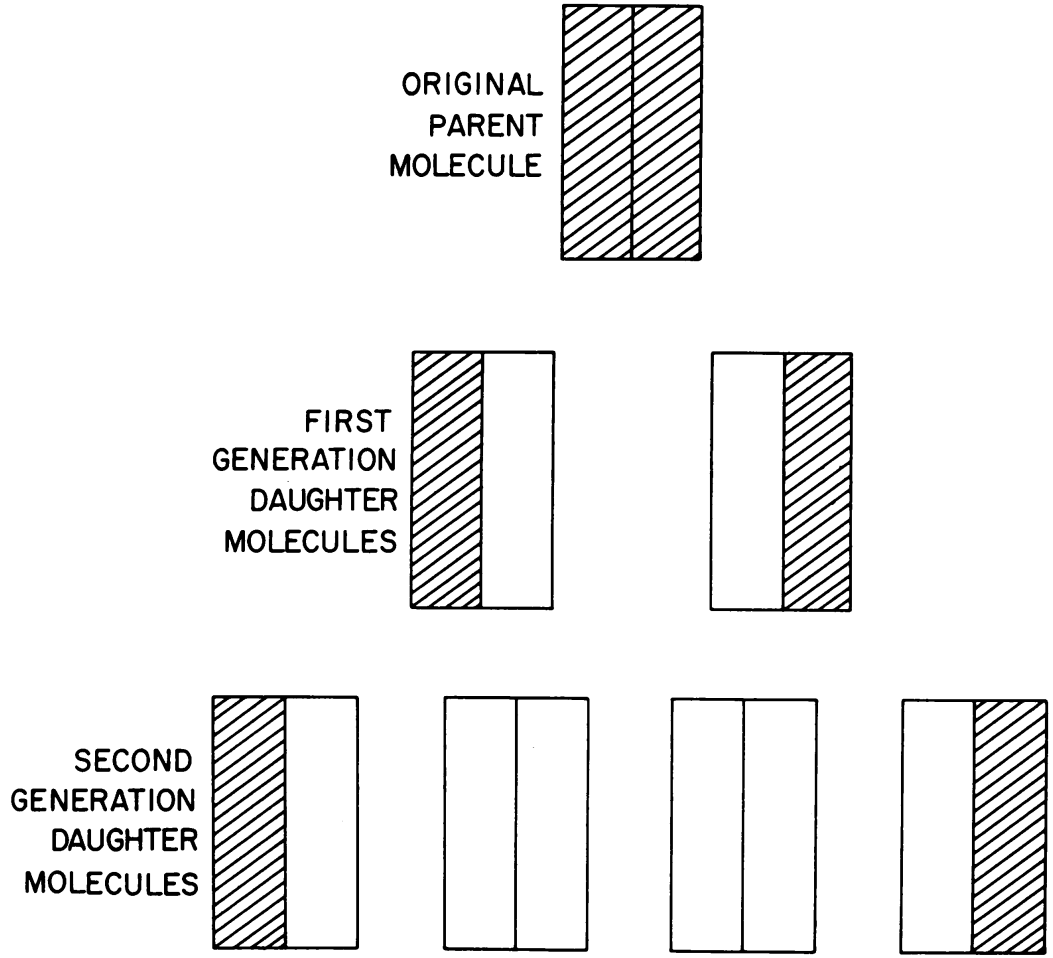

FIG. 5.—Schematic representation of the conclusions drawn in the text from the data presented in Fig. 4. The nitrogen of each DNA molecule is divided equally between two subunits. Following duplication, each daughter molecule receives one of these. The subunits are conserved through successive duplications.

Meselson M. and Stahl FW. (1958) THE REPLICATION OF DNA IN ESCHERICHIA COLI. Proc Natl Acad Sci USA. 44(7):671-82.

Expectations

- Know the importance of DNA replication.
- Know the proposed models of DNA replication.
- Know Meselson and Stahl experiment and the proof that DNA replicates in a semi-conservative fashion.

For a smile