# Sol Spiegelman

Aaron Flynn

Methods and Logic

March 5, 2015

# Biography

- Born December 14th, 1914
- BS in Physics and Mathematics
- Lectured in physics, applied mathematics at Washington University while doing PhD research
- PhD in Cellular Physiology from Washington University
- University of Illinois Professor of Microbiology
- Developed molecular hybridization
- Discovered DNA-dependent RNA polymerase
- Discovered RNA replicating enzyme for Q-beta phage
- Received Lasker Award in 1974
- Director of Cancer Center at Columbia
- Died January 21st, 1983

# Biography

- Famous for formulating daring theories
- Also famous for ardent opposition to the idea of ‘creativity’ in science
- Even before he graduated college, he published controversial research on bacterial genetics

# Bacteriophage Q*β*

- E. coli phage, group IV positive ssRNA E. coli virus
- Three open frames encoding four proteins; A1, A2, CP, Q*β*-replicase.
- Replicase complexes with host S1, EF-Tu, EF-Ts to form RNA polymerase
- In vitro synthesis by Pace and Spiegelman (1966) separated RNA from protein and showed template RNA directs its own synthesis

# *AN EXTRACELLULAR DARWINIAN EXPERIMENT WITH A SELF-DUPLICATING NUCLEIC ACID MOLECULE**

BY D. R. MILLS,† R. L. PETERSON, AND S. SPIEGELMAN

DEPARTMENT OF MICROBIOLOGY, UNIVERSITY OF ILLINOIS, URBANA

*Communicated May 18, 1967*

- "What will happen to the RNA molecules if the only demand made on them is the Biblical injunction, *multiply*, with the biological proviso that they do so as rapidly as possible?"

# Bacteriophage Q$\beta$ RNA

- Spiegelman lab recently identified RNA-dependent RNA polymerases from two bacteriophages, MS-2 and Q$\beta$
- MS-2 and Q$\beta$ replicases are template-specific
- Phage RNA plus replicase plus ribonucleotides leads to more identical RNA, meaning the phage RNA templates itself
- Satisfies definition of a self-duplicating entity

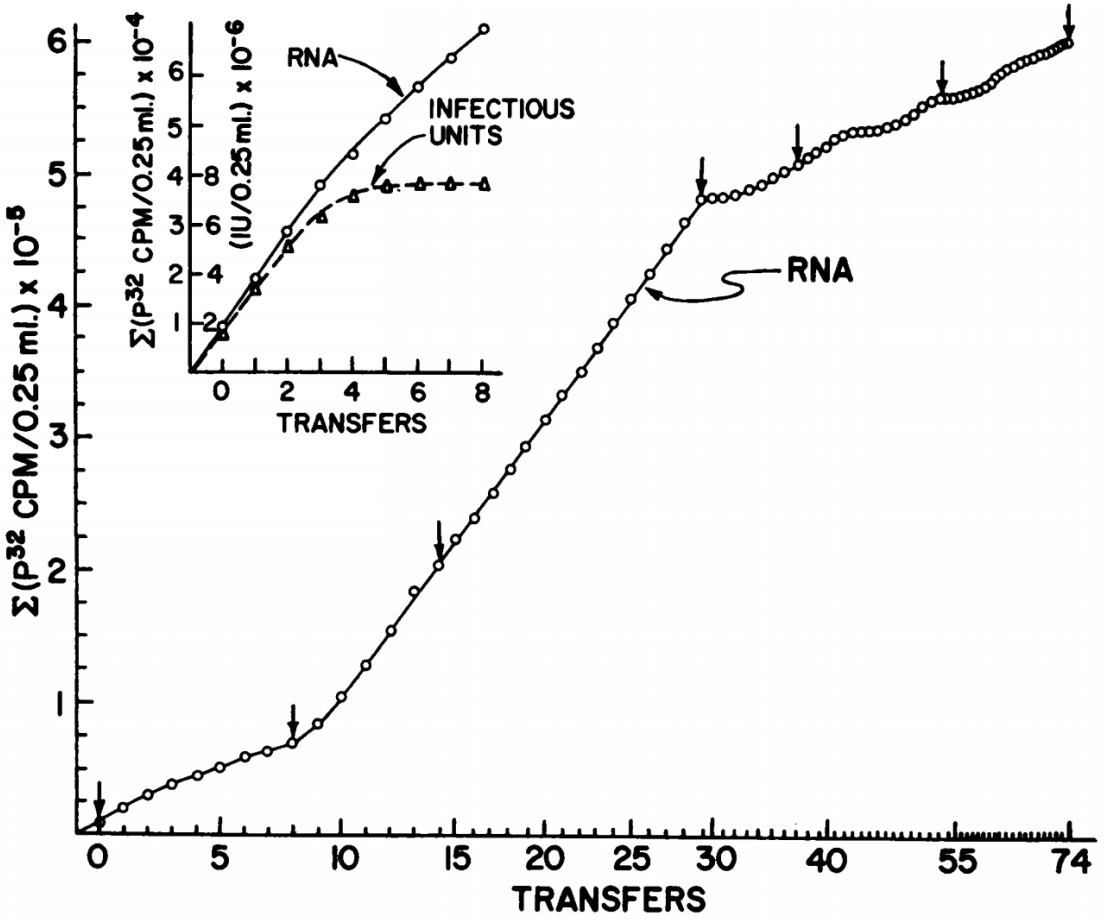
# Bacteriophage Q*β* RNA

- Q*β* bacteriophage replicase previously isolated and shown to template RNA synthesis; RNA-dependent RNA polymerase
- *In vitro* evolution of Q*β* RNA using Q*β* replicase
- RNA which can be replicated faster will have selective advantage

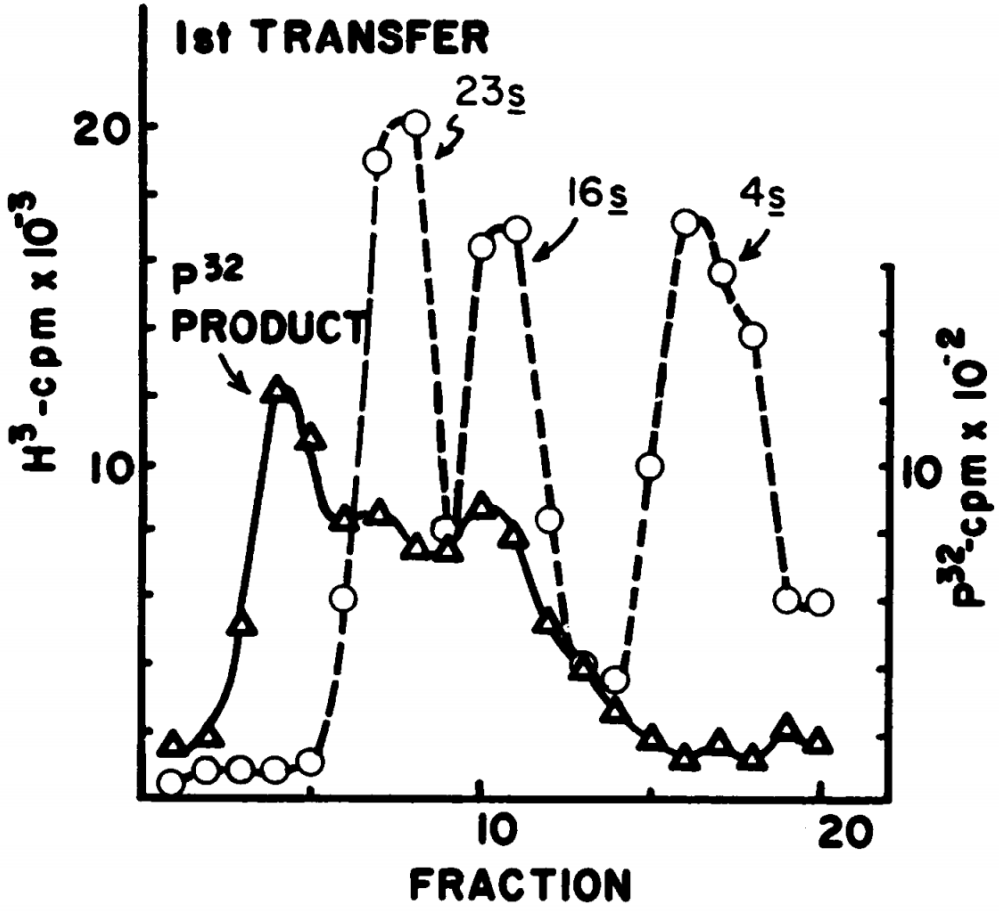
# Approaches

- Measure ribonucleotide addition using $^{32}$P-labeled UTP
- Sedimentation analysis
- Gel electrophoresis
- Electrophoretic mobility
- Replication kinetics

# Serial Transfer Experiment



# Sedimentation analysis



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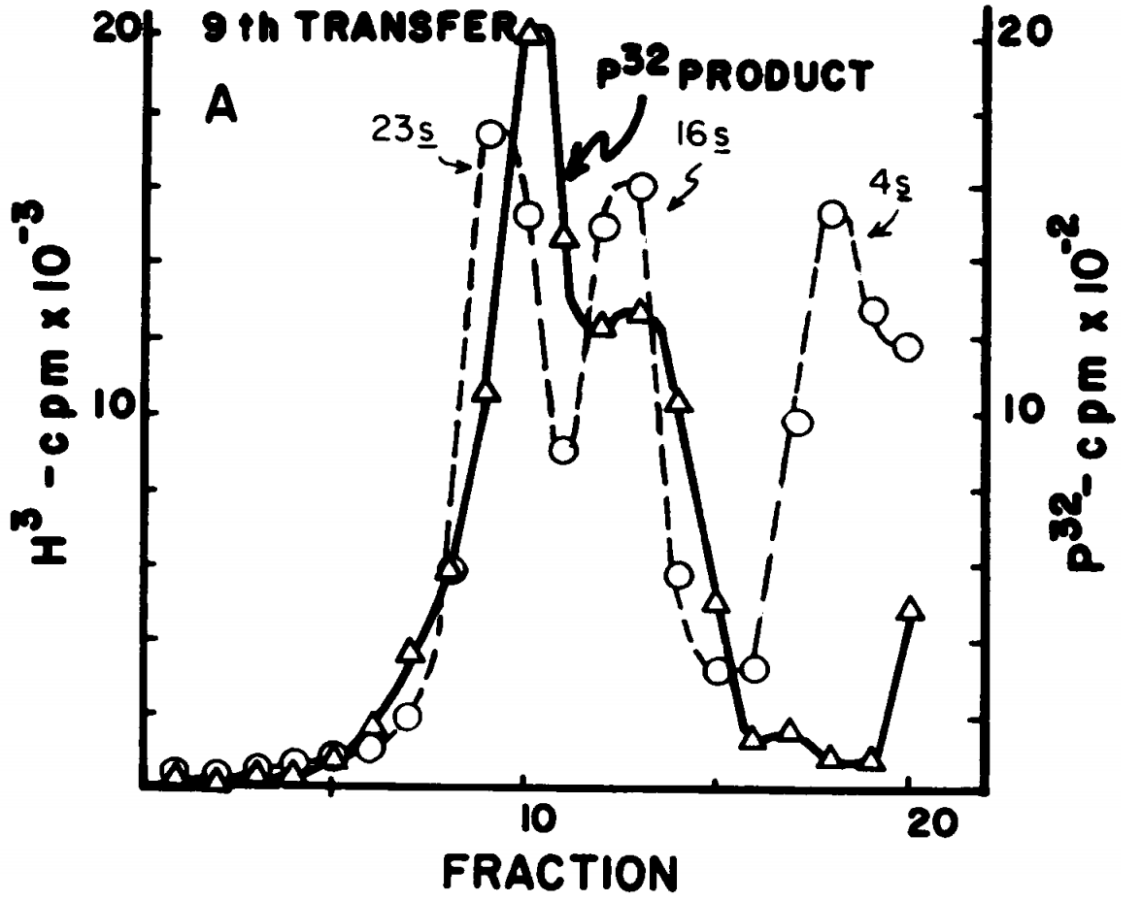

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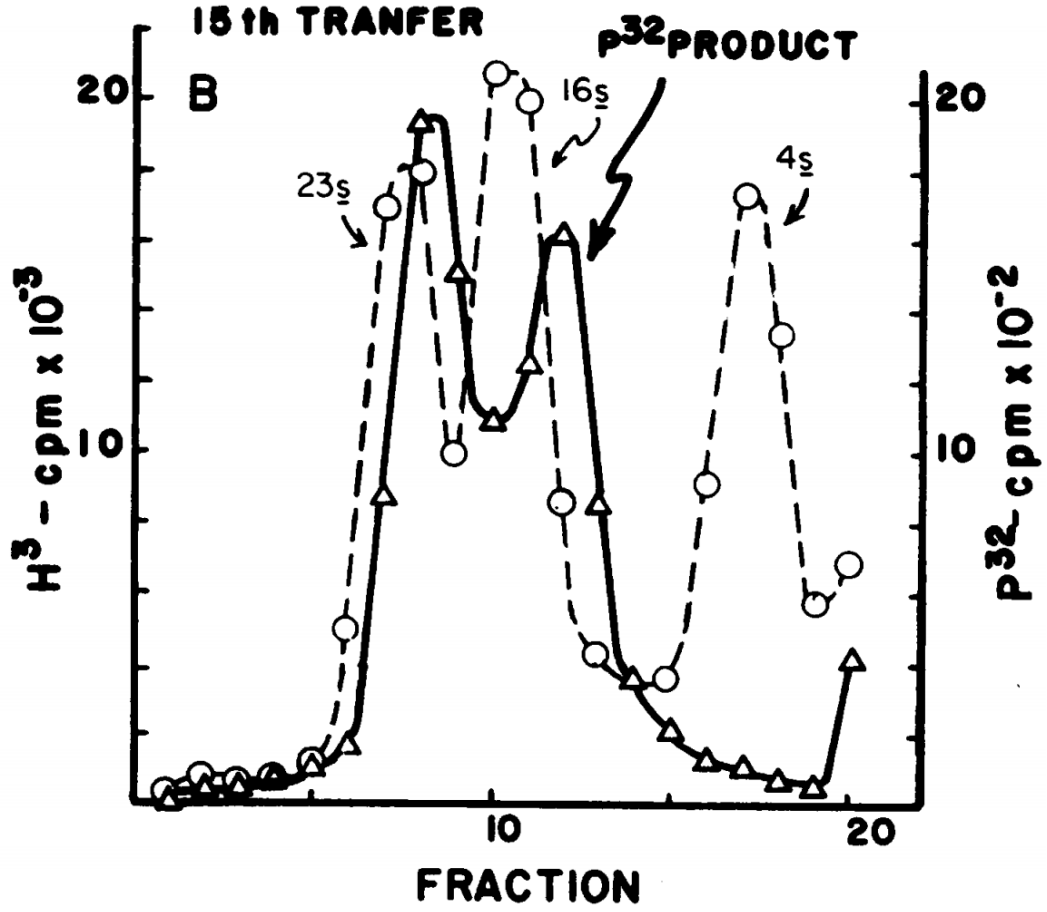

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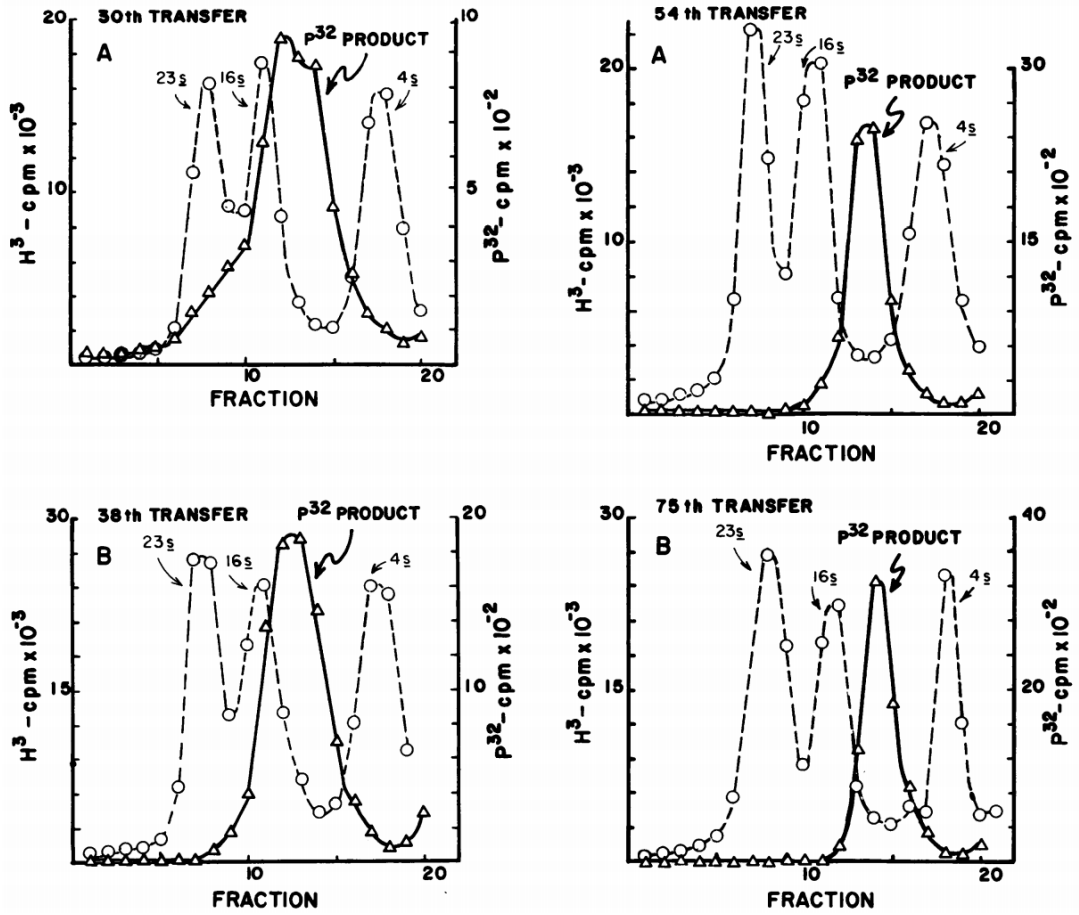
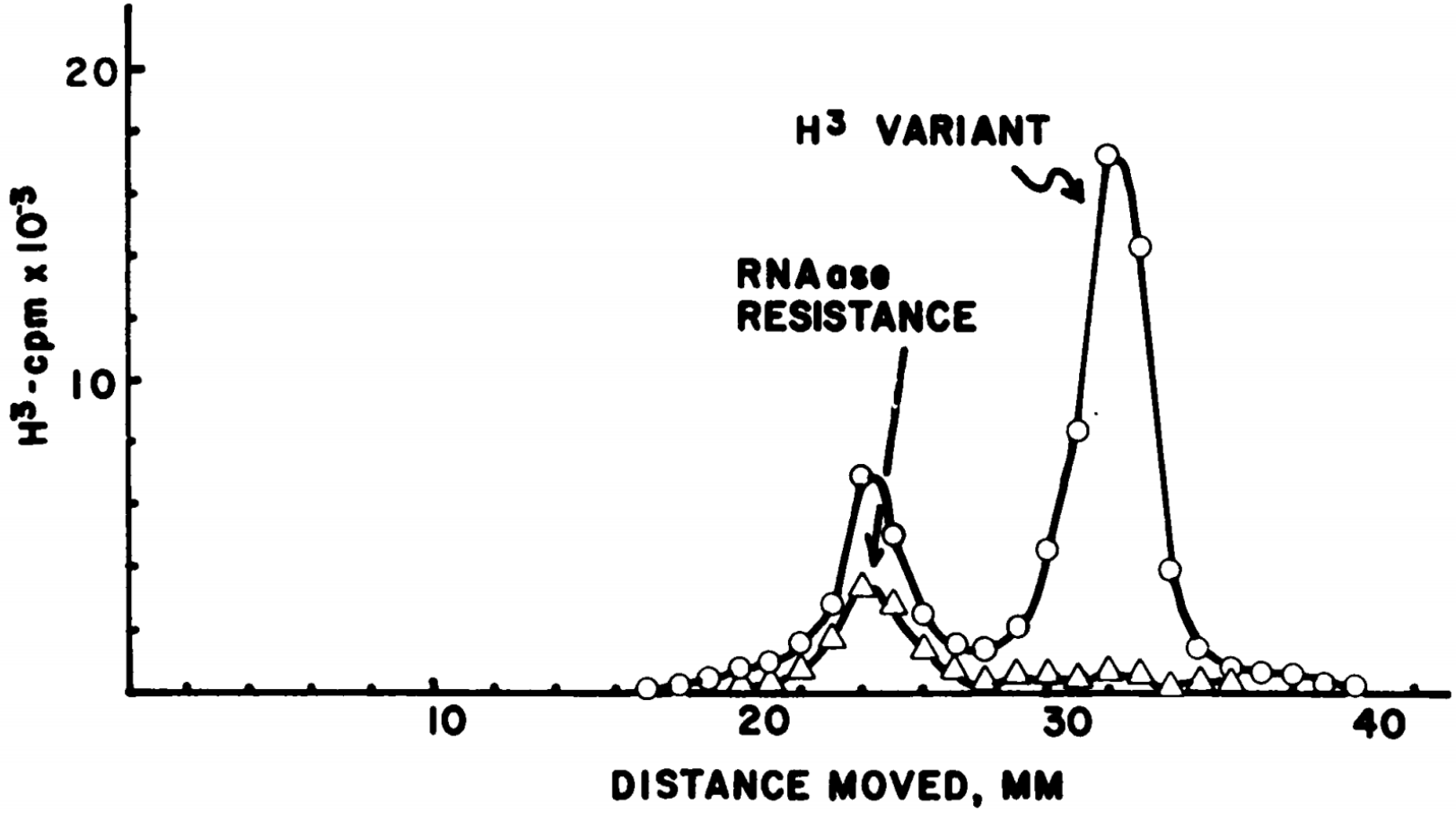
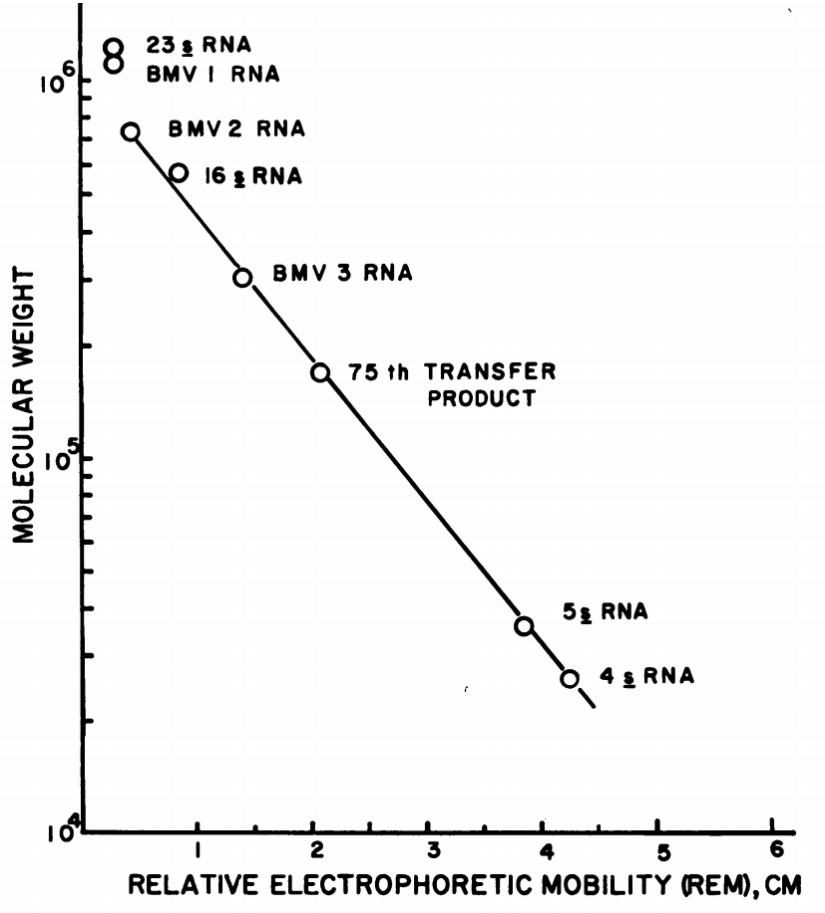


# Gel electrophoresis of 3H CTP-labeled 75$^{th}$ transfer



# Electrophoretic mobility assay



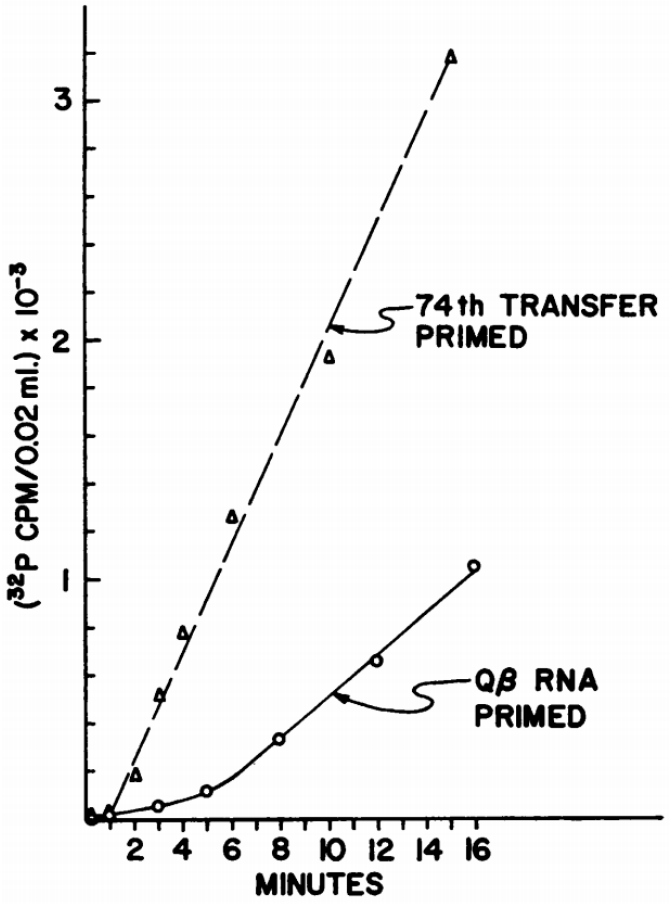
# Base composition analysis

BASE COMPOSITION OF VARIANT RNA

| RNA | C | A | U | G |
|---|---|---|---|---|
| Variant | 22.3 | 19.7 | 29.3 | 28.7 |
| Qβ-RNA-1 | 25.0 | 22.5 | 29.5 | 23.0 |
| Qβ-RNA-2 | 24.7 | 22.1 | 29.1 | 23.7 |

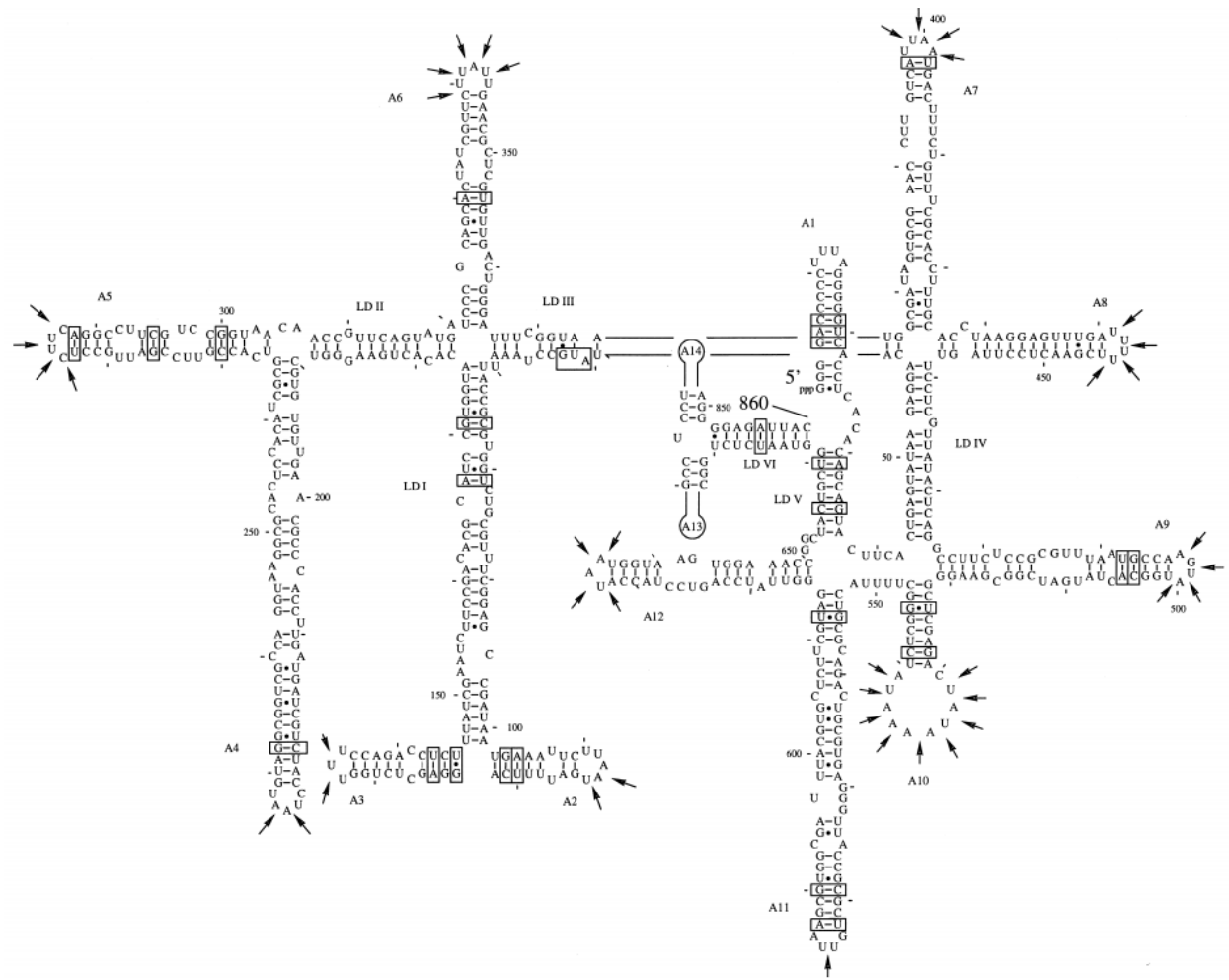
# Replication kinetics

- Variant RNA has shortened lag phase
- Linear portion slope is 2.6 times higher
- Since variant is only 17% the size, growth rate is 15 times higher



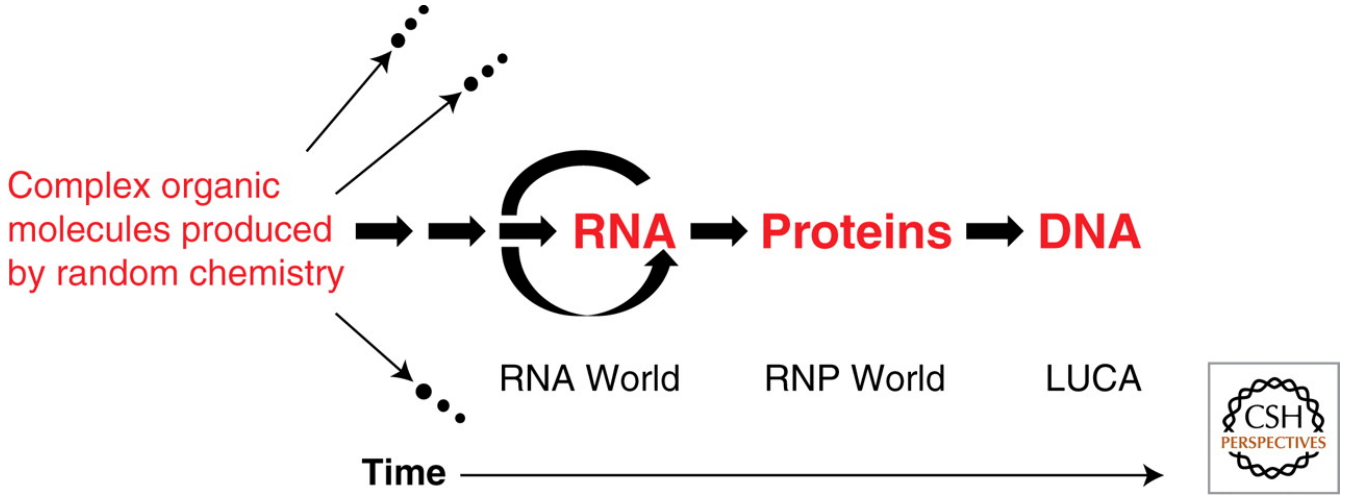
# Conclusions

- Selective pressure on RNA for one trait (*in vitro* replication speed) causes it to discard other traits and unnecessary sequences
- Molecular evolution can be seen *in vitro*


A1
A2
A3
A4
A5
A6
A7
A8
A9
A10
A11
A12
A13
A14
LD I
LD II
LD III
LD IV
LD V
LD VI
5'
860

# RNA World Hypothesis

- Self-replicating RNA molecules are the precursors to all life on earth
- Requires RNA to be informational and catalytic
- Ribozymes discovered in :
  - rRNA
  - Self-splicing introns
  - snRNPs
  - Hammerhead self-cleavage
  - RNA replicase ribozyme
- Regulatory RNA
- Riboswitch RNA
- SELEX
- **Evolving RNA**



# Sources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