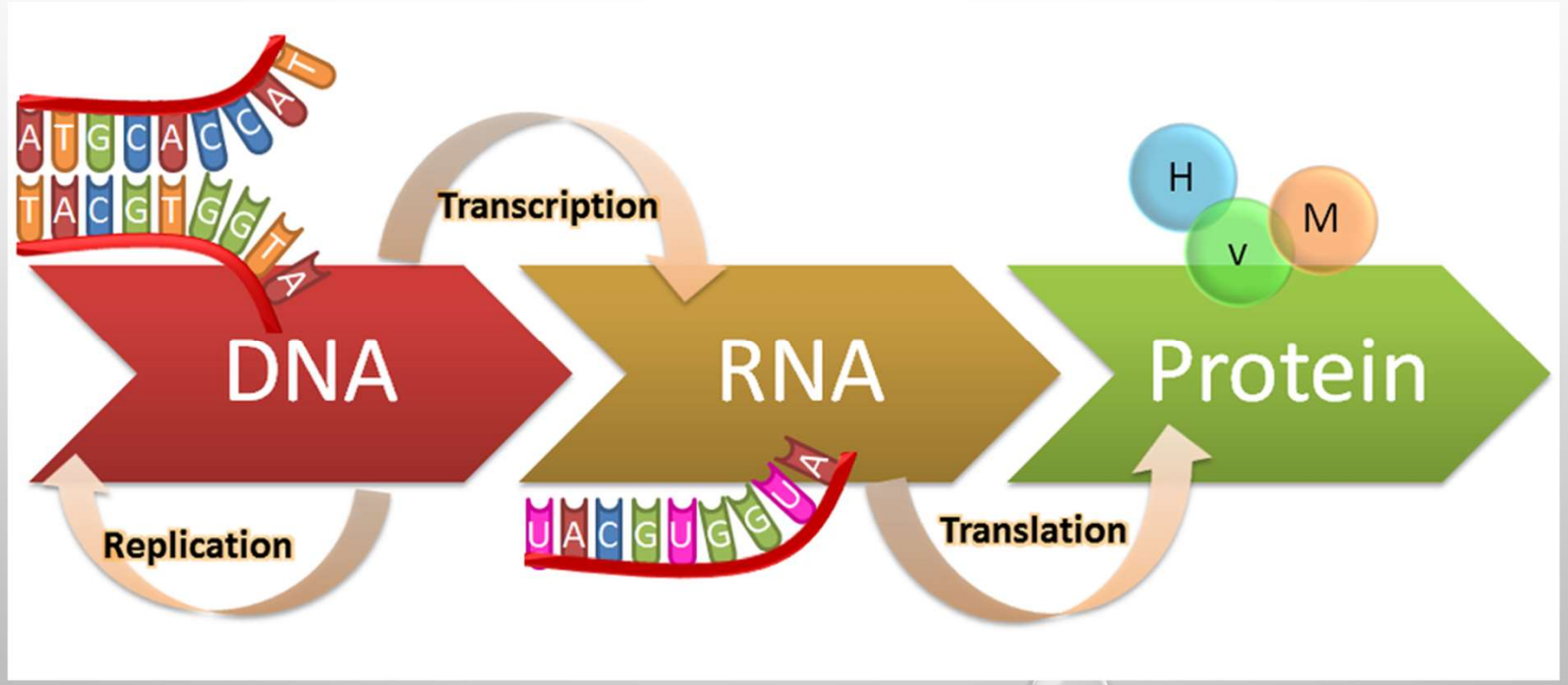
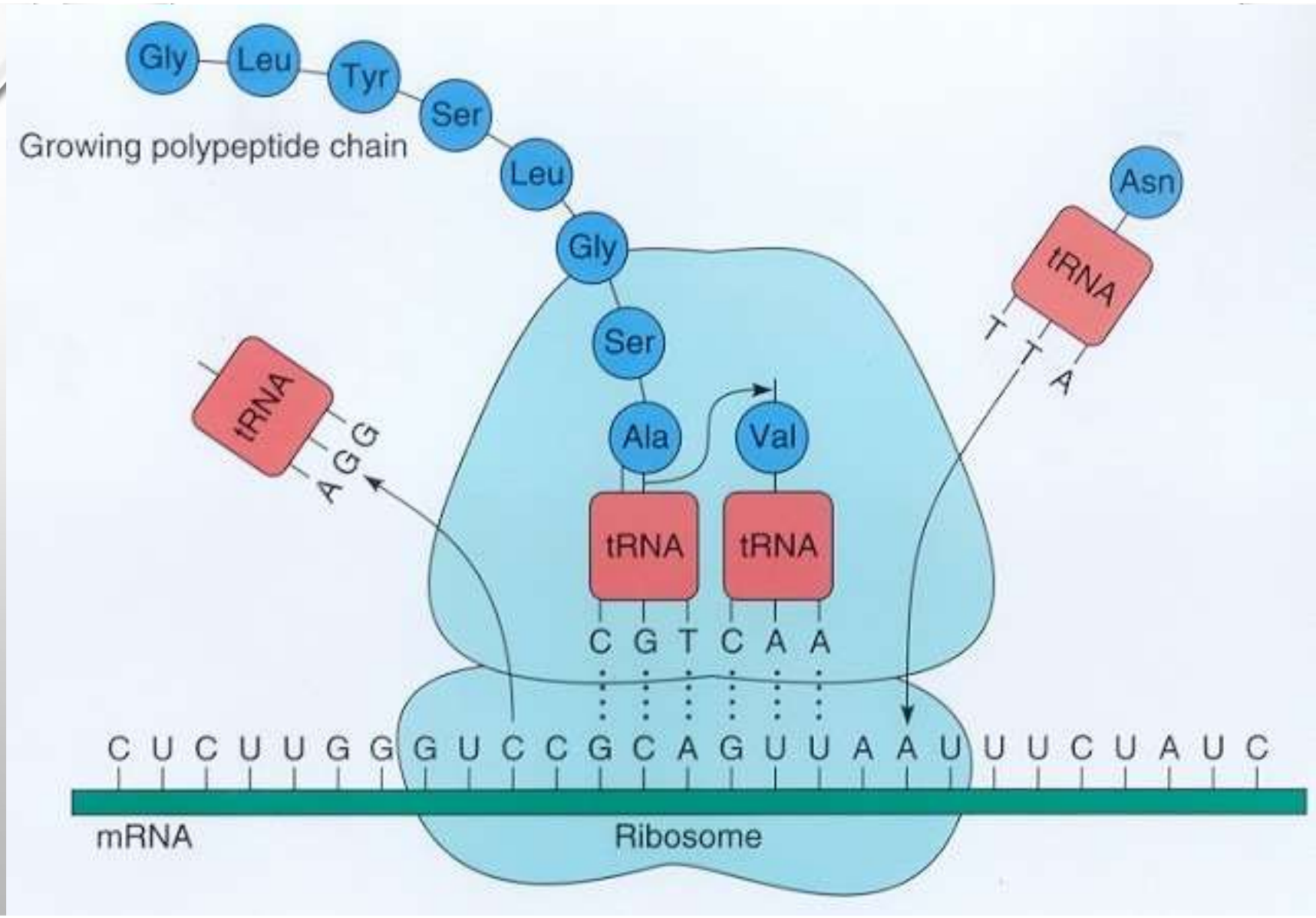
THE MOLECULAR HISTORY OF LIFE

FALL 2023

THE INFORMATION HIGHWAY: FROM CODE TO CELLULAR FUNCTION




Gly
Leu
Tyr
Ser
Leu
Gly
Ser
Ala
Val
Growing polypeptide chain
tRNA
A G G
tRNA
tRNA
tRNA
T T A
Asn
C G T C A A
C U C U U G G G U C C G C A G U U A A U U U C U A U C
mRNA
Ribosome

RNA AS FUNCTION

- IT CAN CLEAVE OTHER RNA MOLECULES AT SPECIFIC SITES
- IT IS ESSENTIAL IN THE PROCESSING AND MATURATION OF OTHER RNA MOLECULES
- IT IS INVOLVED IN THE MAINTENANCE OF TELOMERES
- IT CAN REGULATE GENE EXPRESSION

- **AND IT IS AT THE CENTER OF PROTEIN SYNTHESIS IN THE RIBOSOME**

REMEMBER SESSION 1

- **MOLECULAR REQUIREMENTS FOR LIVING SYSTEMS:**
- 1) MAINTAINING STRUCTURE → METABOLISM
- 2) INFORMATION
- 3) REPLICATION

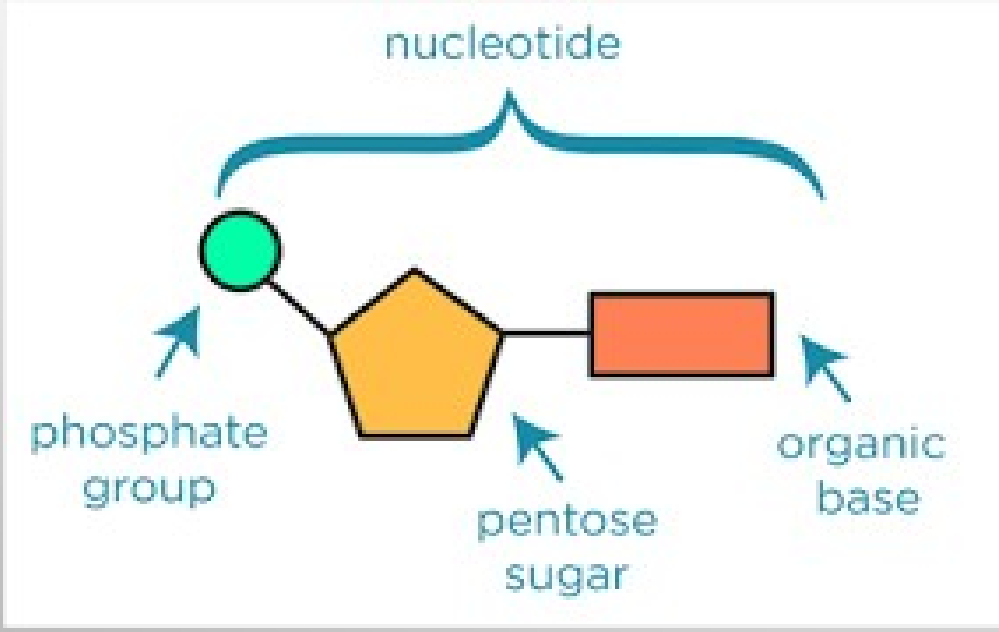
- **ALL THE ABOVE FUNCTIONS (INFORMATION, REPLICATION AND METABOLISM) CAN BE ACCOMPLISHED BY RNA**

- **WELCOME TO THE "RNA WORLD"**
- CAVEAT EMPTOR: THIS IS **HYPOTHETICAL**

AN "RNA WORLD" SCENARIO

- JACK SZOSTAK
- NOBEL IN CHEMISTRY IN 2009 (FOR WORK ON TELOMERES AND THE RNA-CONTAINING ENZYME THAT PROTECTS THEM)
- HARVARD AND U. CHICAGO SINCE 2022
- PROPOSED A SIMPLE 2-COMPONENT SYSTEM FOR THE EVOLUTION OF THE "RNA WORLD"
- THE TWO KEY COMPONENTS OF A PRIMITIVE CELL WOULD BE A SELF-REPLICATING NUCLEIC ACID GENOME, AND A SELF-REPLICATING BOUNDARY STRUCTURE

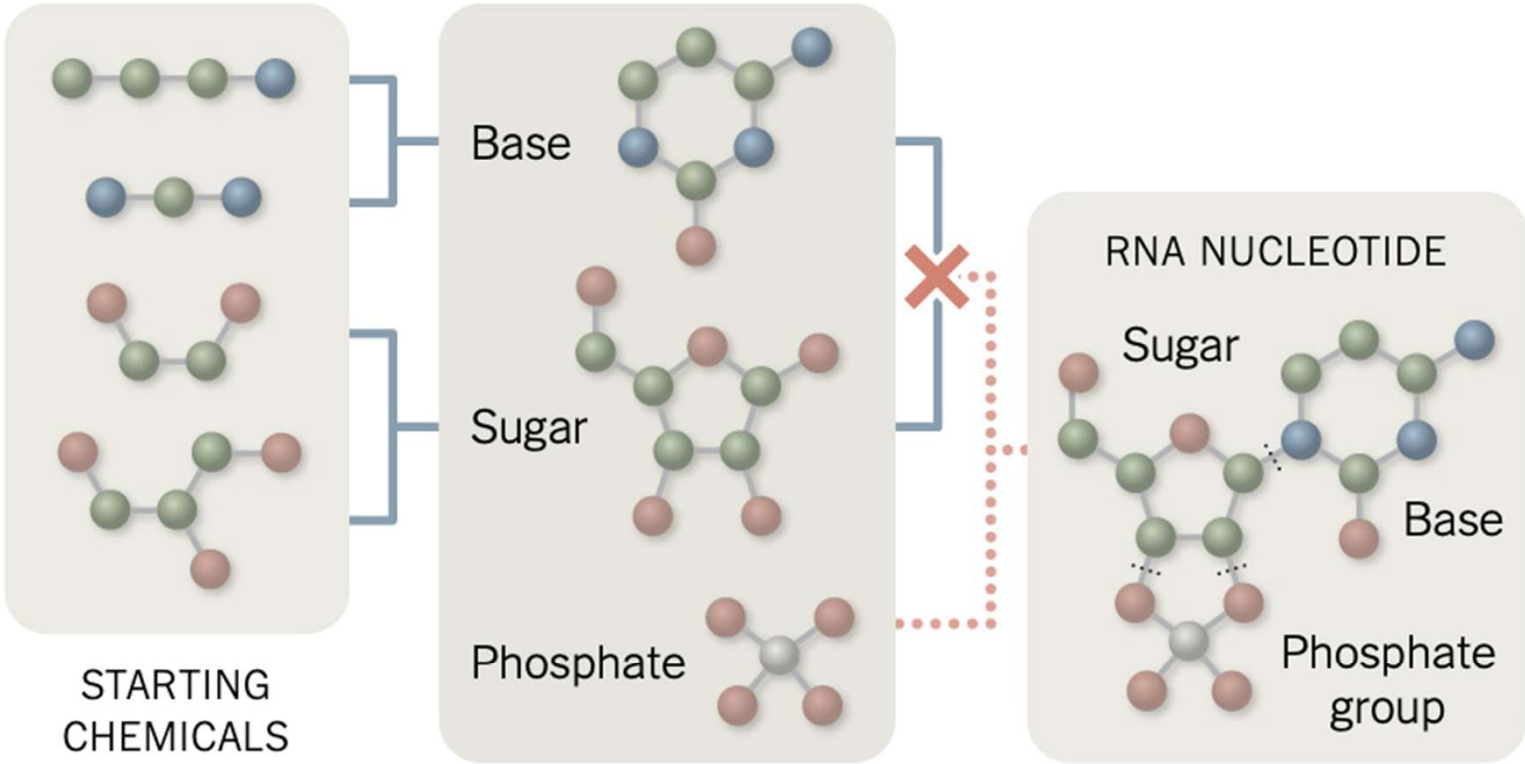
BUILDING AN RNA WORLD



- WE NEED TO ASCERTAIN THE POSSIBILITY THAT THE COMPONENTS OF RNA (4 NUCLEOTIDES: A, G, C, AND U) COULD HAVE BEEN FORMED IN A PREBIOTIC WORLD

PREVIOUS ATTEMPTS to explain how RNA formed focused on its three components: a phosphate group, a base and a sugar molecule (ribose).

But chemists could not find a natural way to join the base and sugar to form RNA.



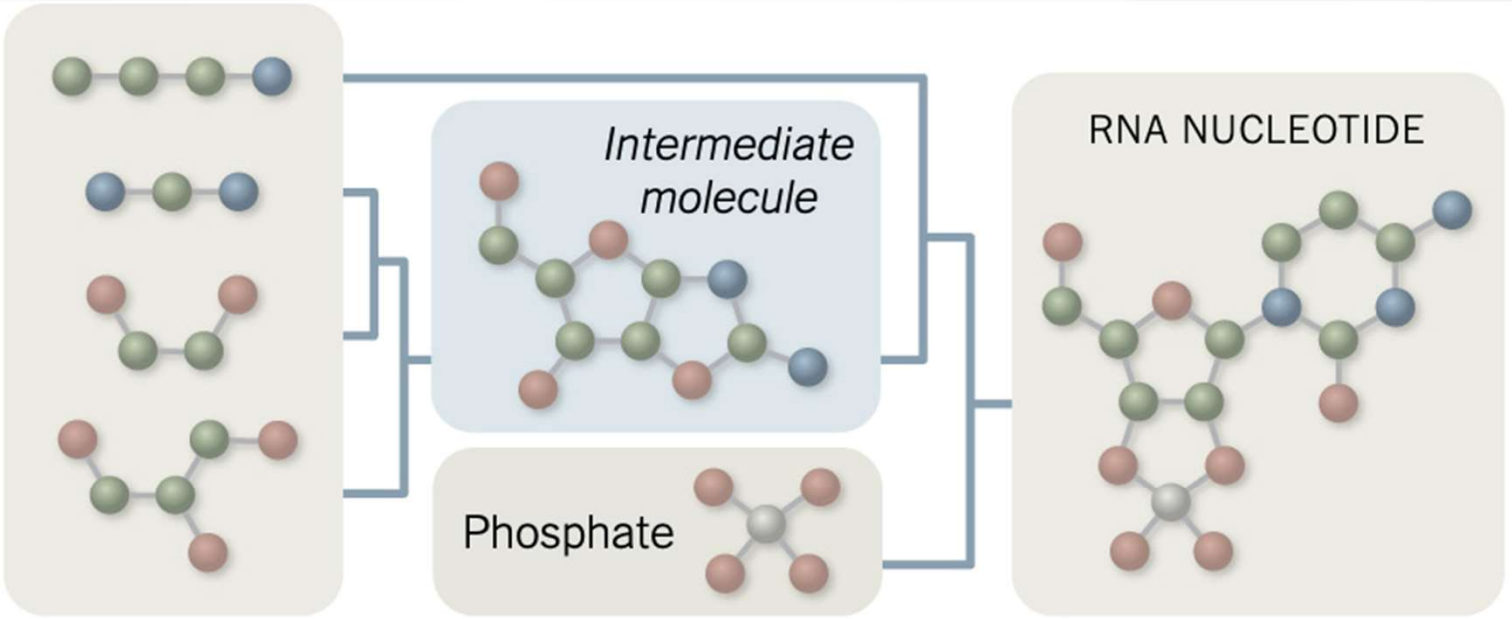
THINKING (AND SYNTHESIZING) OUTSIDE THE BOX

- IN 2019, RESEARCHERS FROM CAMBRIDGE AND MUNICH UNIVERSITY, PUBLISHING IN *SCIENCE*:
- **UNIFIED PREBIOTICALLY PLAUSIBLE SYNTHESIS OF PYRIMIDINE AND PURINE RNA RIBONUCLEOTIDES**
- THE SYNTHESIS REQUIRED ONLY SIMPLE COMPOUNDS (AS THOSE WE TALKED ABOUT IN SESSION 1) AND WAS DRIVEN BY CYCLES OF WET-DRY CONDITIONS, SIMILAR TO PRESENT CYCLES OF DROUGHT AND RAIN
- THESE CHEMISTRIES COULD HAPPEN AT HIGHER OR LOWER TEMPERATURES (ALBEIT MORE SLOWLY)

THINKING (AND SYNTHESIZING) OUTSIDE THE BOX

A NEWER MODEL combines the same starting chemicals in a different order, avoiding the base and sugar molecules.

An RNA molecule can emerge from naturally forming intermediate molecules, part sugar and part base.



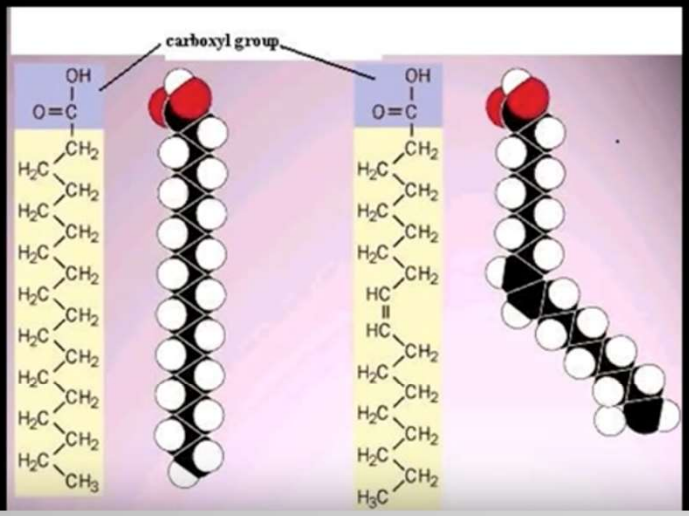
BUILDING RNA CHAINS

- RESEARCHERS FROM MCMASTER UNIVERSITY, CANADA, AND MAX PLANCK INSTITUTE, GERMANY
- PUBLISHED IN PNAS, 2017
- WARM LITTLE PONDS (WLPS) ARE EXCELLENT CANDIDATE ENVIRONMENTS FOR POLYMERIZATION OF NUCLEOTIDES AND FORMATION OF RNA CHAINS
- IN SIMULATIONS OF WET/DRY CYCLES IN WLPS, POLYMERIZATION OF NUCLEOTIDES INTO CHAINS GREATER THAN 300 LINKS WAS OBSERVED
- IN SIMULATIONS OF DEEP SEA HYDROTHERMAL VENT ENVIRONMENTS ONLY YIELDED POLYMERS OF 20 MONOMERS

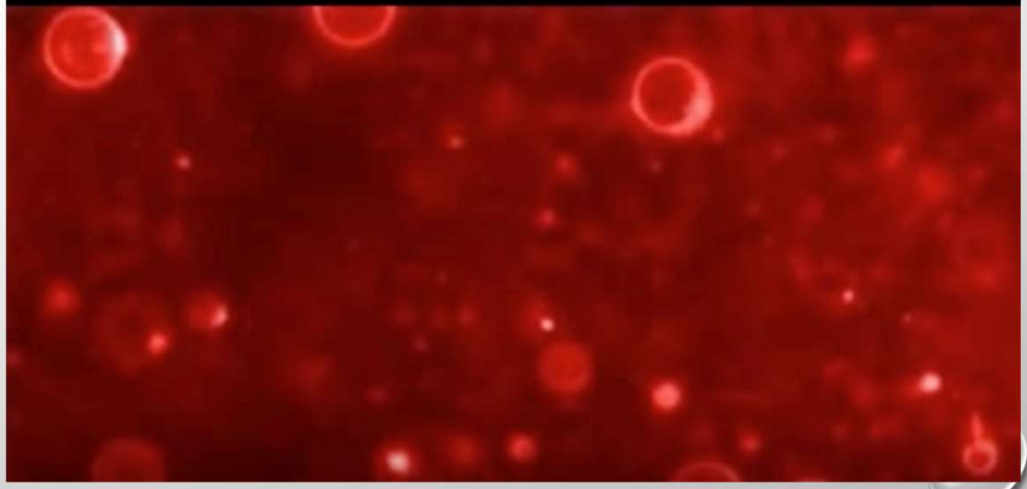
OVERCOMING DIFFUSION:

ISOLATING FROM THE ENVIRONMENT – THE MAKINGS OF A 'MEMBRANE'

The pre-biotic environment contained many simple fatty acids

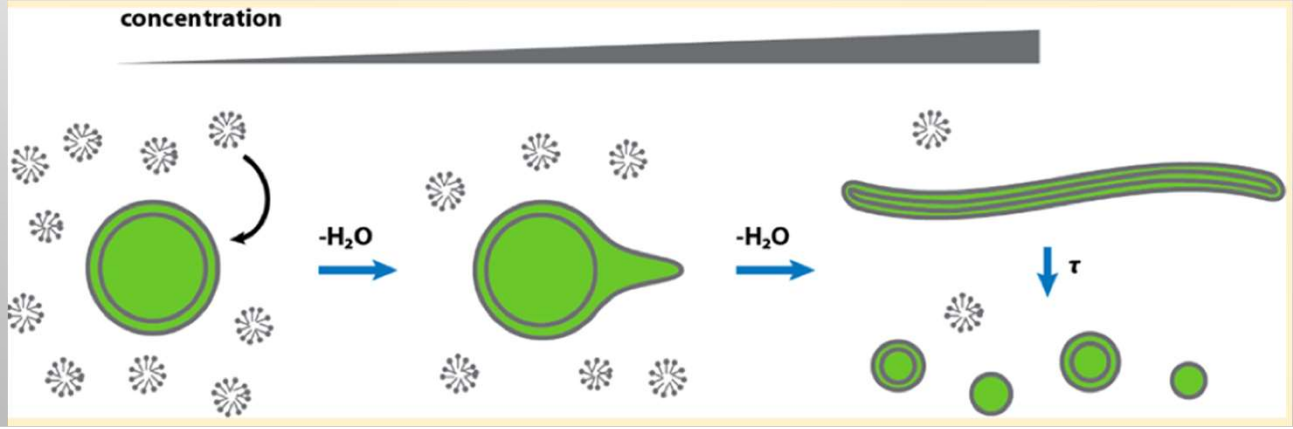


SPONTANEOUSLY form stable vesicles.

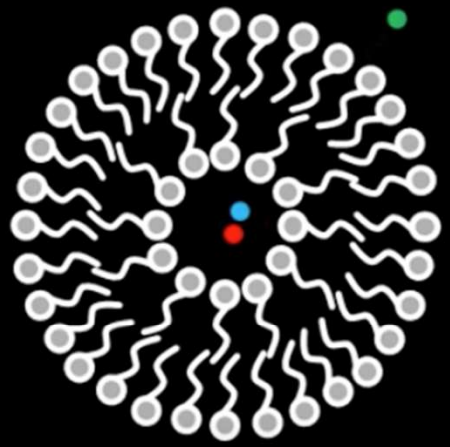


VESICLES CAN GROW AND DIVIDE

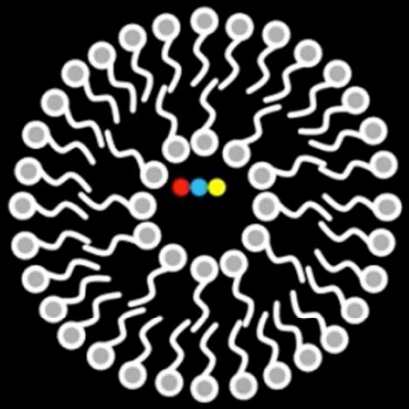
- CONSIDER A SMALL WARM POND, CONTAINING DILUTE FATTY ACIDS, ALONG WITH OTHER ORGANIC COMPOUNDS
- **CYCLES OF EVAPORATION AND REHYDRATION**
- EVAPORATION, DRIVEN BY SOLAR OR GEOTHERMAL HEAT, WOULD LEAD TO PROGRESSIVE CONCENTRATION OF THE DISSOLVED SOLUTES AND THUS TO VESICLE GROWTH
- THIS GROWTH COULD RESULT IN FILAMENTOUS VESICLES, WHICH ARE FRAGILE
- EVEN WEAK SHEARING FORCES (THINK RAIN, FALLING ON THE POND) WOULD RESULT IN DIVISION INTO DAUGHTER VESICLES



Our fatty acid vesicles are permeable to nucleotide monomers, but not polymers.

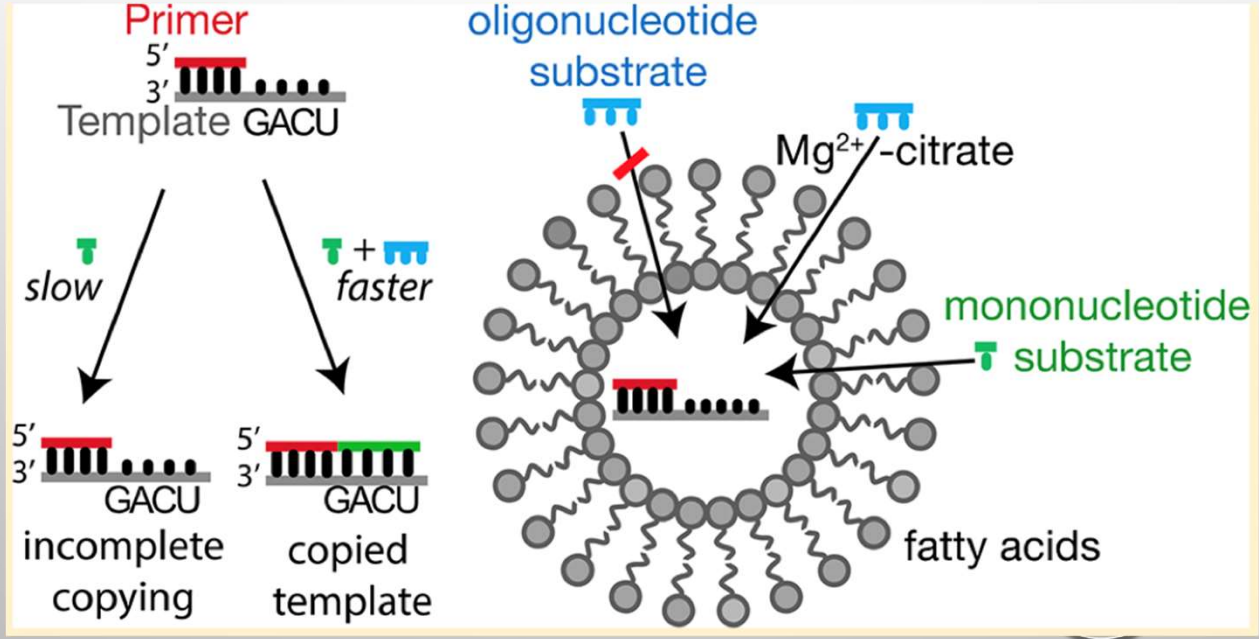


Once spontaneous polymerization occurs within the vesicle, the polymer is trapped.



HOW TO REPLICATE RNA INSIDE VESICLES

- IN 2018, SZ0STAK'S GROUP DEMONSTRATED THAT SHORT RNA MOLECULES, UP TO 4 NUCLEOTIDES LONG, CAN ALSO CROSS INTO THE FATTY ACID VESICLES
- THESE SHORT STRETCHES OF RNA CAN PAIR WITH THE RANDOM CHAINS GENERATED INSIDE THE VESICLE AND ACCELERATE THE RATE OF COPYING



A SIMPLE TWO-COMPONENT SYSTEM (LIPIDS AND RNA)

- SPONTANEOUSLY FORMS IN THE PRE-BIOTIC ENVIRONMENT
- THE SYSTEM CAN EAT (INCORPORATE NUCLEOTIDES AND SHORT RNA SEQUENCES FROM THE ENVIRONMENT)
- IT CAN GROW, CONTAIN INFORMATION AND REPLICATE
- THEREFORE, IN PRINCIPLE, THE SYSTEM CAN **EVOLVE**

EVOLVING EARLY GENOMES

- EARLY RNA INSIDE THE FATTY ACID VESICLES WERE COMPLETELY RANDOM
- THEREFORE, THEY CONTAINED **NO** INFORMATION
- IT WAS THEIR ABILITY TO SPONTANEOUSLY REPLICATE, IRRESPECTIVE OF SEQUENCE, THAT DROVE GROWTH AND DIVISION OF THE VESICLES
- BUT THE SPONTANEOUS REPLICATION WILL BE "IMPERFECT" AND WILL INTRODUCE VARIATION
- AND, ANY MUTATION THAT INCREASED THE RATE OF RNA REPLICATION WOULD BE SELECTED FOR

- **THIS IS DARWINIAN EVOLUTION**

FROM THEORY TO PRAXIS –
CONFIRMATION OF RNA EVOLUTION

- **EVOLUTIONARY TRANSITION FROM A SINGLE RNA REPLICATOR TO A MULTIPLE REPLICATOR NETWORK -** UNIVERSITY OF TOKYO. PUBLISHED IN *NATURE* ON 2022
- FIRST EMPIRICAL EVIDENCE THAT SIMPLE SYSTEMS CAN LEAD TO THE EMERGENCE OF COMPLEX LIFELIKE SYSTEMS
- SYNTHESIZED AN RNA MOLECULE THAT REPLICATES
- THE SINGLE RNA SPECIES EVOLVED INTO A COMPLEX SYSTEM COMPRISING FIVE TYPES OF RNAS
- TIMED ANALYSIS OF SAMPLES REVEALED THAT THERE WAS FIRST A MULTITUDE OF CHANGES THAT EVENTUALLY COALESCED INTO FIVE DISTINCT TYPES
- RESULTS DEMONSTRATE AN EVOLUTIONARY TRANSITION SCENARIO FROM A SINGLE COMMON ANCESTOR TO A MULTI-MEMBERED NETWORK
- **THIS IS DARWINIAN EVOLUTION**

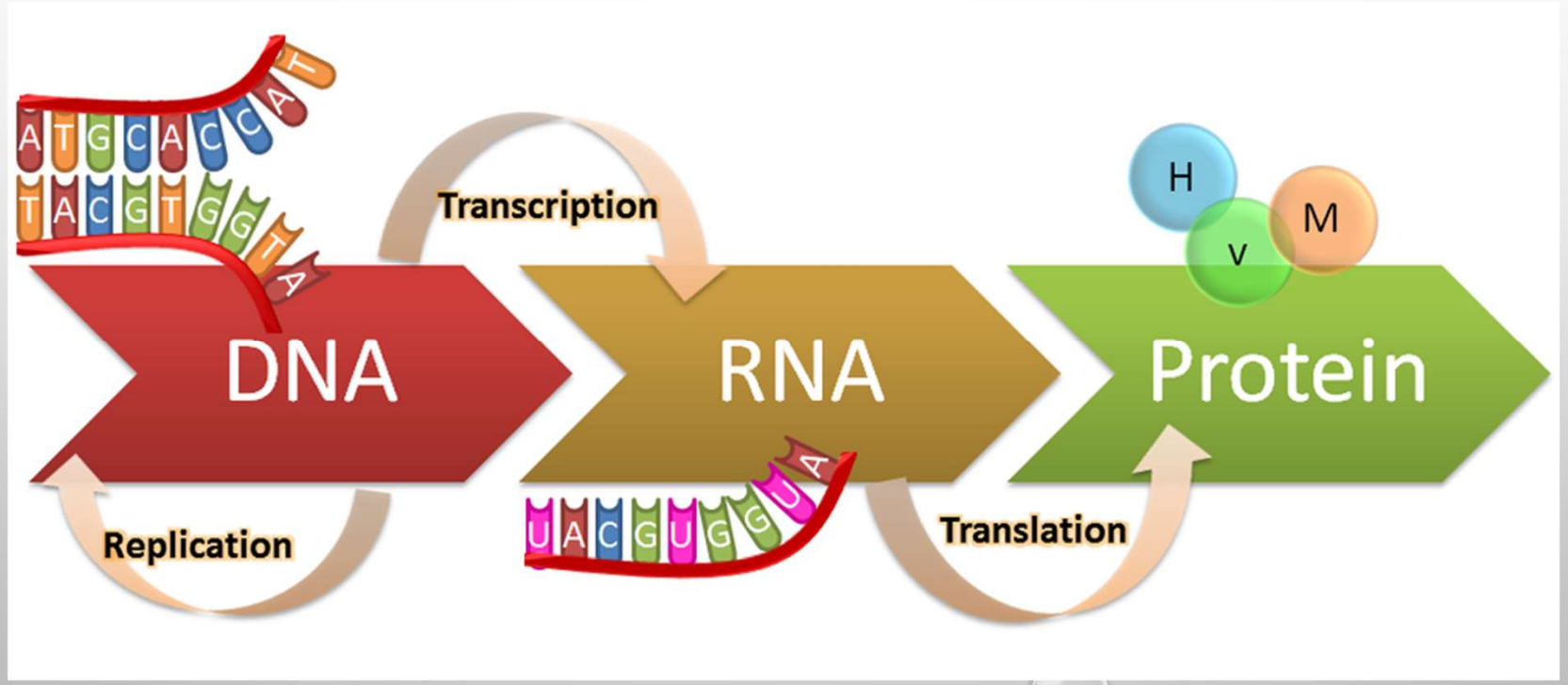
POSSIBLE BENEFICIAL MUTATIONS

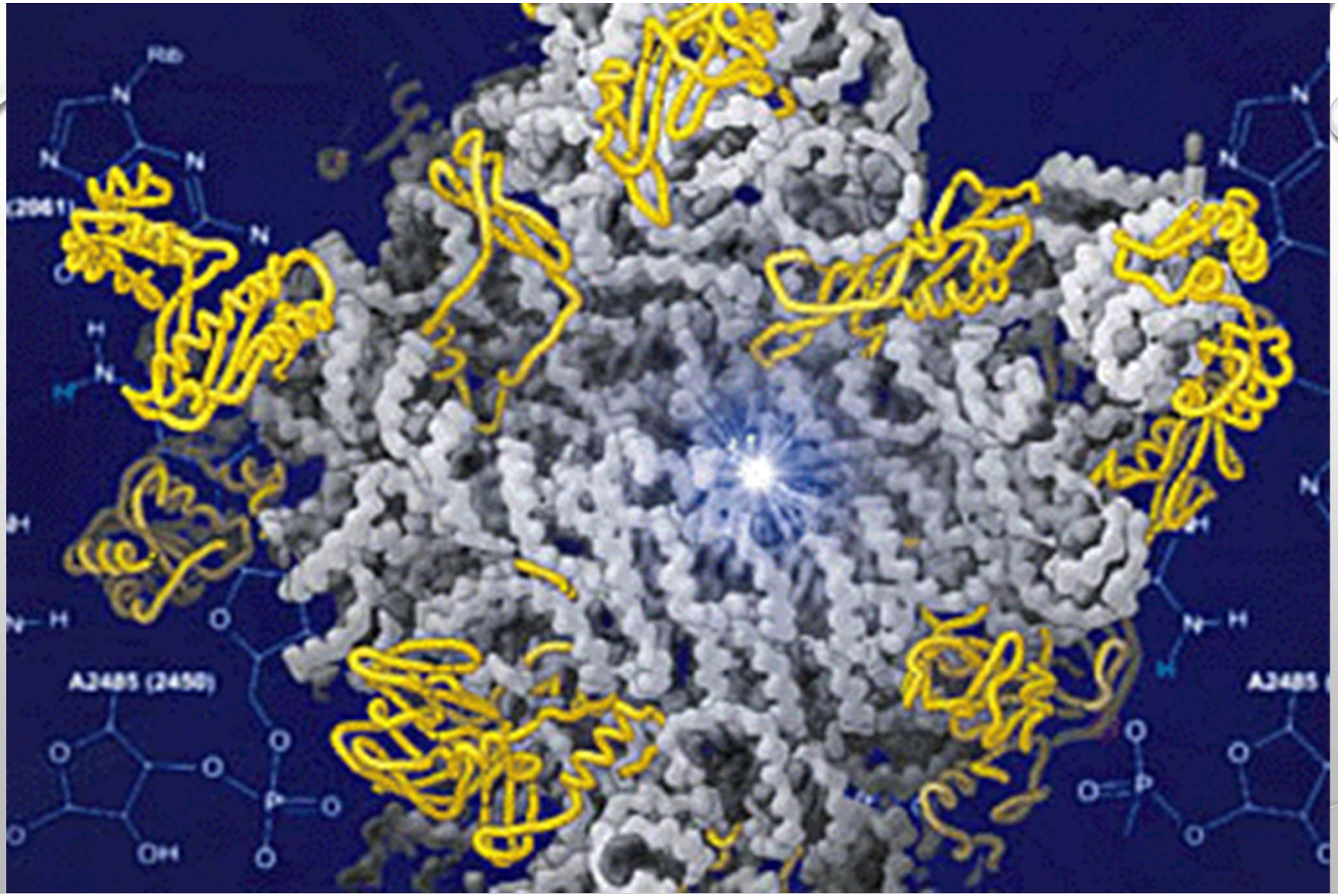
- CHANGES IN THE SEQUENCE OF RNA TO CONTAIN ONLY THE MOST COMMON NUCLEOTIDES
- FORM SEQUENCES THAT ARE STABLE, YET SEPARATE EASILY SO THEY CAN REPLICATE
- EVOLVE RNA STRUCTURES THAT CAN SHOW SOME ADVANTAGEOUS BIOCHEMICAL ACTIVITY
- ACTIVITIES COULD INCLUDE:
  - ENHANCED REPLICATION ABILITY
  - SYNTHESIZE LIPIDS TO GENERATE THEIR OWN LIPIDS FOR GROWING MEMBRANES
  - SYNTHESIS OF NUCLEOTIDES
  - LIGATE MOLECULES
  - REGULATE THEIR OWN EXPRESSION

BRINGING PROTEINS INTO THE RNA WORLD

- IN THE 1970S, ORGEL AND COLLEAGUES REPORTED POTENTIALLY PREBIOTIC ROUTES TO FORMING AMINO ACID-BRIDGED DINUCLEOTIDES
- IN 2021, SZOSTAK'S GROUP SHOWED THAT THESE AMINO ACID-DINUCLEOTIDES CAN ACCELERATE RNA COPYING BY ORDERS OF MAGNITUDE
- AS AN ADDED BONUS, THE REACTION HAPPENS IN CONDITIONS THAT GREATLY ACCELERATE VESICLE GROWTH

THE INFORMATION HIGHWAY: FROM CODE TO CELLULAR FUNCTION

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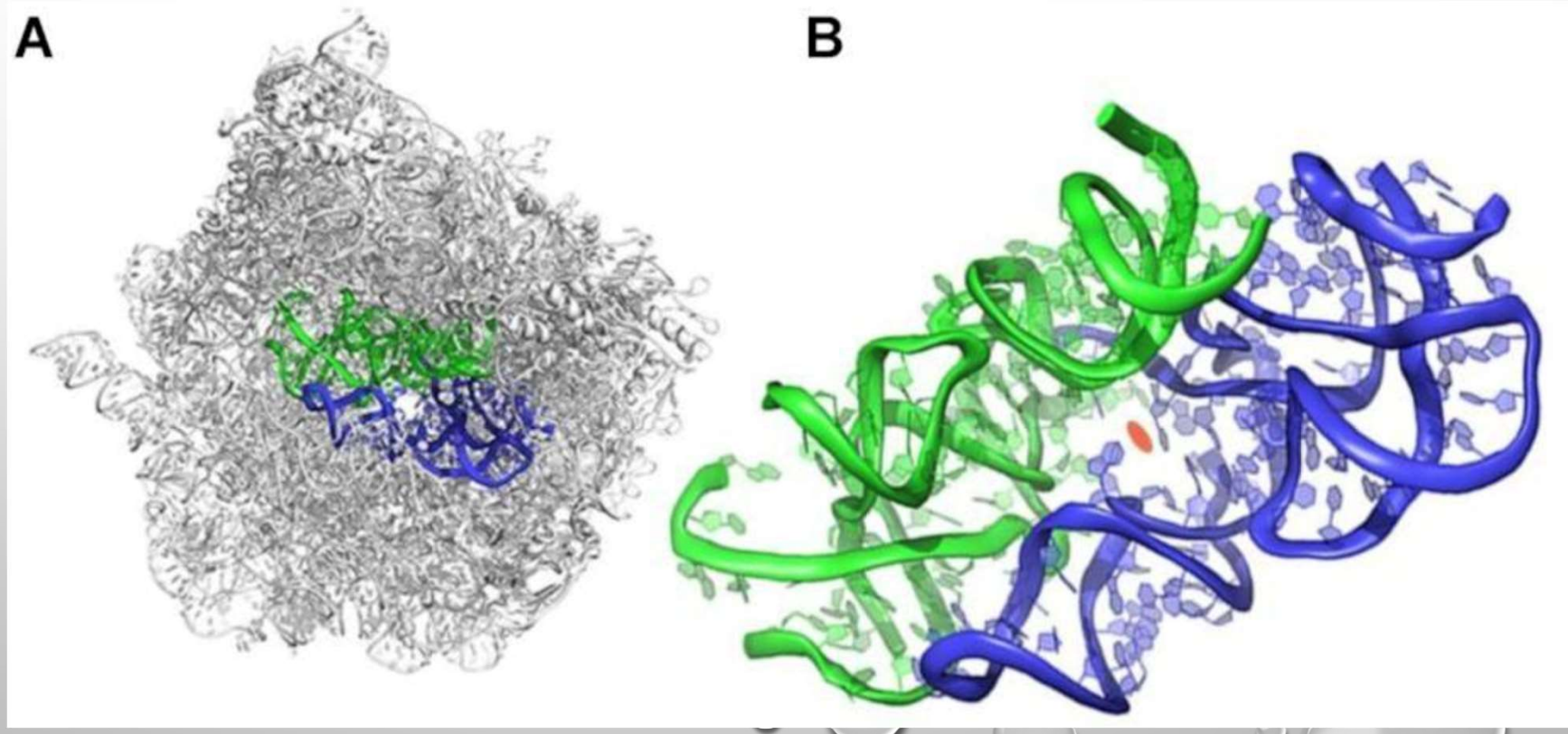
LITTLE BY LITTLE : THE PROTO-RIBOSOME

- **ORIGIN OF LIFE: PROTO-RIBOSOME FORMS PEPTIDE BONDS AND LINKS RNA AND PROTEIN DOMINATED WORLDS**
- ADA YONAH ET AL., *NUCLEIC ACIDS RES.* 2022



THE (NOT TOO MODEST) PROPOSAL

- IDENTIFIED A UNIVERSAL INTERNAL SMALL RNA POCKET-LIKE SEGMENT IN THE LARGE SUBUNIT OF THE RIBOSOME, WHERE THE CATALYTIC ACTIVITY LINKING AMINO ACIDS RESIDES
- THIS REGION IS ONLY 6% OF THE ENTIRE LARGE SUBUNIT
- THIS STRUCTURE IS UNIVERSALLY CONSERVED
- THEY POSITED THIS POCKET IS A VESTIGE OF THE PRIMORDIAL RIBOSOME
- ASCERTAINED THAT TRNAS, AS WELL AS SMALLER ANALOGS , CAN BIND THIS POCKET AT TWO SITES
- THE THREE-DIMENSIONAL ARRANGEMENT POSITIONS THE SUBSTRATES IN A SPATIAL ORIENTATION OPTIMAL FOR JOINING TWO AMINO ACIDS
- PROPOSED THAT THIS POCKET, WHICH IS STILL IMBEDDED IN THE MODERN RIBOSOME, IS ACTUALLY THE PROTO-RIBOSOME, NAMELY THE REMNANT OF AN ANCIENT BARE-BONES PROTEIN-MAKING MACHINERY

THE EXPERIMENT

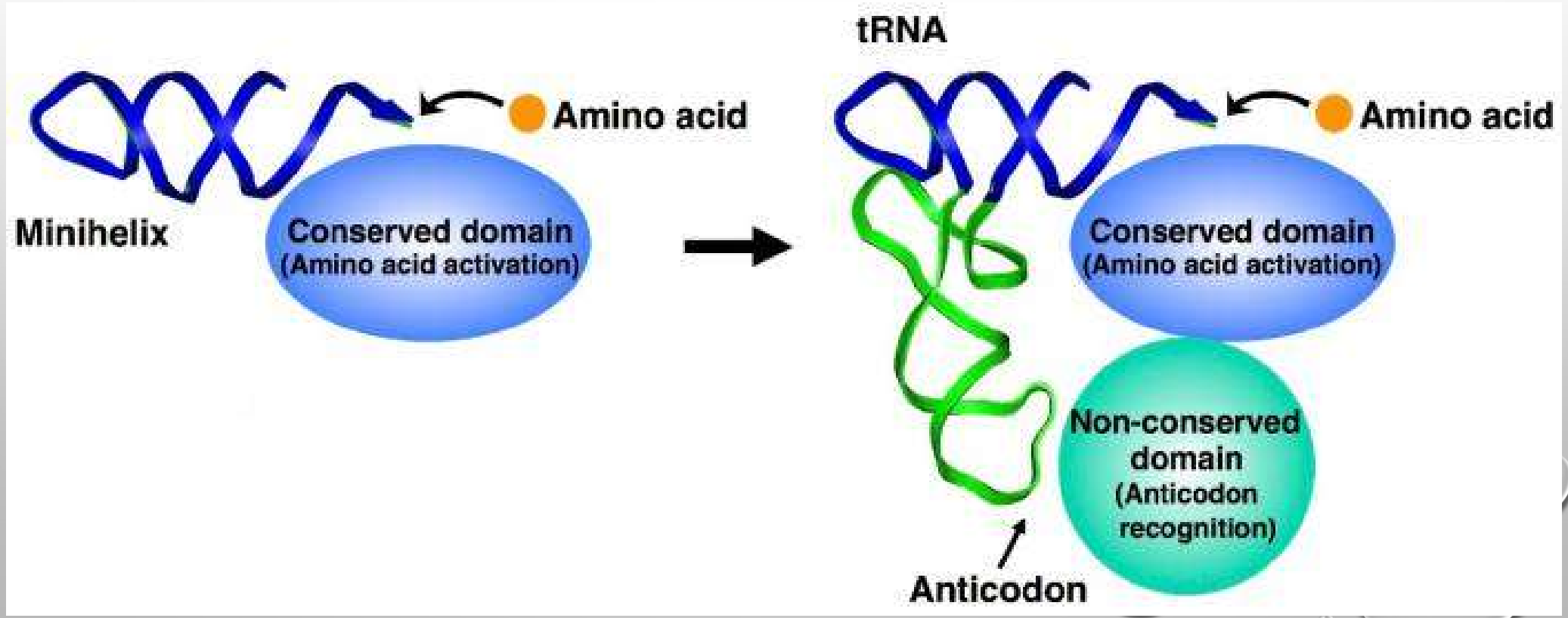
- DESIGN A MINIMAL RNA THAT MIMICS THE POCKET-LIKE STRUCTURE OF THE CONTEMPORARY RIBOSOME
- ASSESS WHETHER THEY CAN CATALYZE THE BONDING OF AMINO ACIDS
- **AND THEY DID!**
- THE CATALYTIC ACTIVITY WAS LIMITED (NOT NEARLY WHAT A PRESENT RIBOSOME CAN DO)
- THE CONSTRUCTS COULD FORM PEPTIDE BONDS AT DIVERSE TEMPERATURES → THE PROTO-RIBOSOME COULD MAINTAIN ITS ACTIVITY IN THE PREBIOTIC WORLD WHERE TEMPERATURE AND ENVIRONMENTAL CONDITIONS WERE PROBABLY QUITE ERRATIC

- THE PROTO-RIBOSOME GROWS BY ACCRETION (THE KLUDGE FACTOR)

CEMENTING TRANSLATION

- SO FAR, WE CAN ENVISION SCENARIOS, WITH SOME EXPERIMENTAL EVIDENCE, FOR THE EVOLUTION OF AN ANCESTRAL TRANSLATION MACHINERY (JOINING AMINO ACIDS)
- IN THE BEGINNING, IN THE PROTO-RIBOSOME, THE LINKING OF AMINO ACIDS WOULD HAVE BEEN RANDOM, I.E. **NOT** CODED
- HOW DO WE GET TO CODED PROTEINS?
- THE EVOLUTION OF TRNAS RESULTS IN THE COUPLING OF DECODING AND AMINO ACID POSITIONING FOR EFFECTIVE LINKAGE

EVOLUTION OF TRNAS



CEMENTING TRANSLATION (CONT.)

- TRNAS ENABLE THE COUPLING OF SPECIFIC AMINO ACIDS TO SPECIFIC RNA SEQUENCES
- TRNAS BRIDGE THE SITE OF DECODING OF AN RNA AND THE SYNTHESIS OF THE ENCODED PROTEIN

- THIS IS THE UNIVERSALLY CONSERVED **GENETIC CODE**

- **THIS ENABLES INFORMATION SHARING, ACCELERATING THE RATE OF EVOLUTION!**

AT THIS POINT, WE HAVE A **LIVING SYSTEM**

- WITH RNA AS THE GENETIC MATERIAL
- PROTEINS DOING MOST OF THE WORK

- LIMITATIONS TO AN RNA GENOME:
- CHEMICALLY NOT VERY STABLE
- THEREFORE, RNA GENOMES CAME IN MANY PIECES → A NIGHTMARE TO DIVIDE EQUALLY AMONG PROGENY
- THERE ARE LIMITS TO THE GENETIC COMPLEXITY

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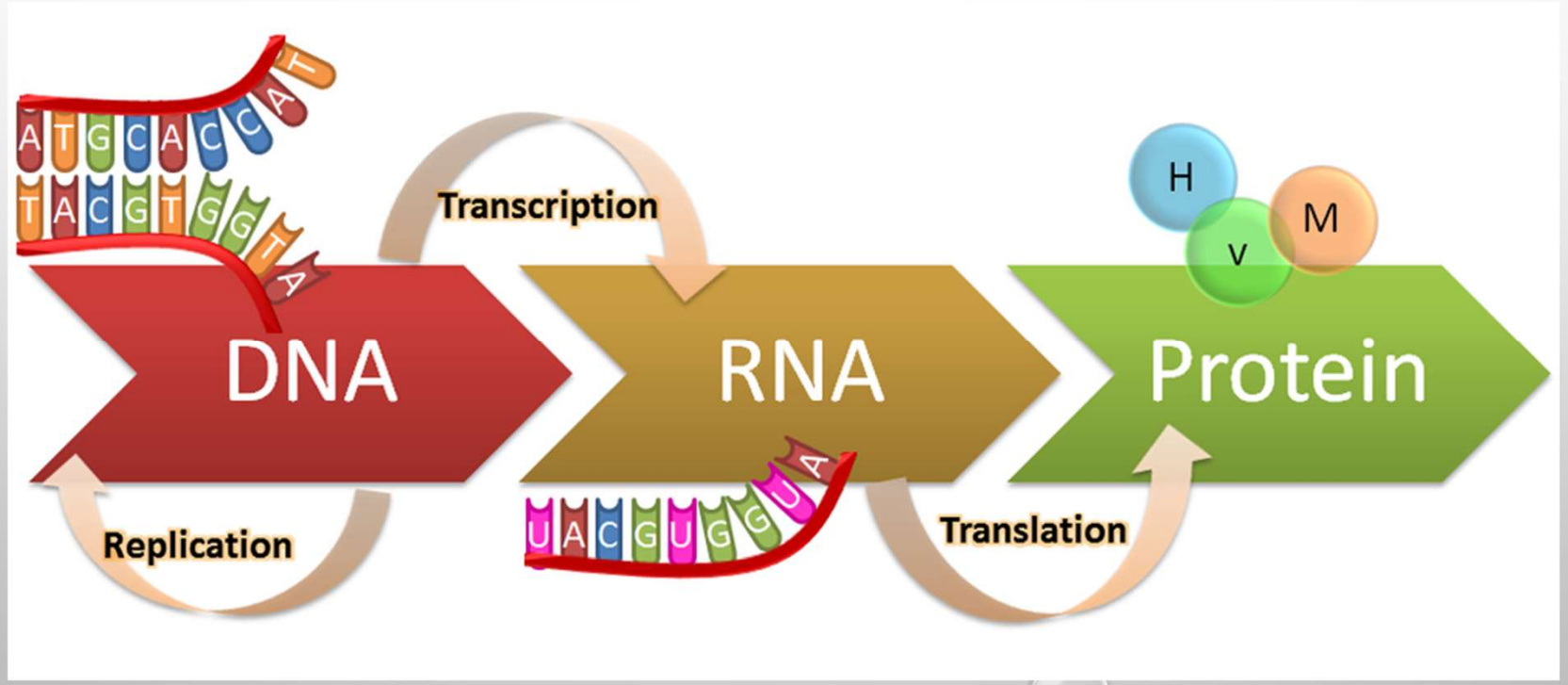

THE ROUTE TO STABLE DNA GENOMES

- THE TRANSITION FROM RNA GENOMES TO DNA GENOMES WAS LIKELY A GRADUAL PROCESS INVOLVING HYBRID RNA–DNA MOLECULES
- OVER TIME, THE INFORMATION WOULD HAVE BEEN STORED IN THE FORM OF DNA, WHICH IS MUCH MORE STABLE THAN DNA
- DETERMINANTS OF STABILITY: DOUBLE HELICAL STRUCTURE, BASE PAIRING, SUGAR, THYMINE INSTEAD OF URACIL

URACIL IN RNA, THYMINE IN DNA

- IN DNA, URACIL IS REPLACED BY THYMIDINE
- WHY?

- CYTOSINE CAN DEAMINATE SPONTANEOUSLY TO PRODUCE URACIL

- BUT C PAIRS WITH G
- IF IT CHANGES TO U, IT NOW PAIRS WITH A → **MUTATION**