# Understanding biological factories to fuel drug discovery

*OR*

# How I was schooled by The RIBOSOME


Jamie Williamson, PhD

*Professor*

*Department of Integrative Structural and Computational Biology*


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at Scripps Research

# Crick’s Central Dogma

DNA

→ RNA

→ Protein

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# Crick's Central Dogma

DNA

Transcription

*Cellular Processes*

RNA

Translation

Protein

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# Crick's Central Dogma

DNA

Transcription

RNA polymerase

RNA

Translation

The RIBOSOME

Protein

*Cellular Processes*

*Cellular Machines*

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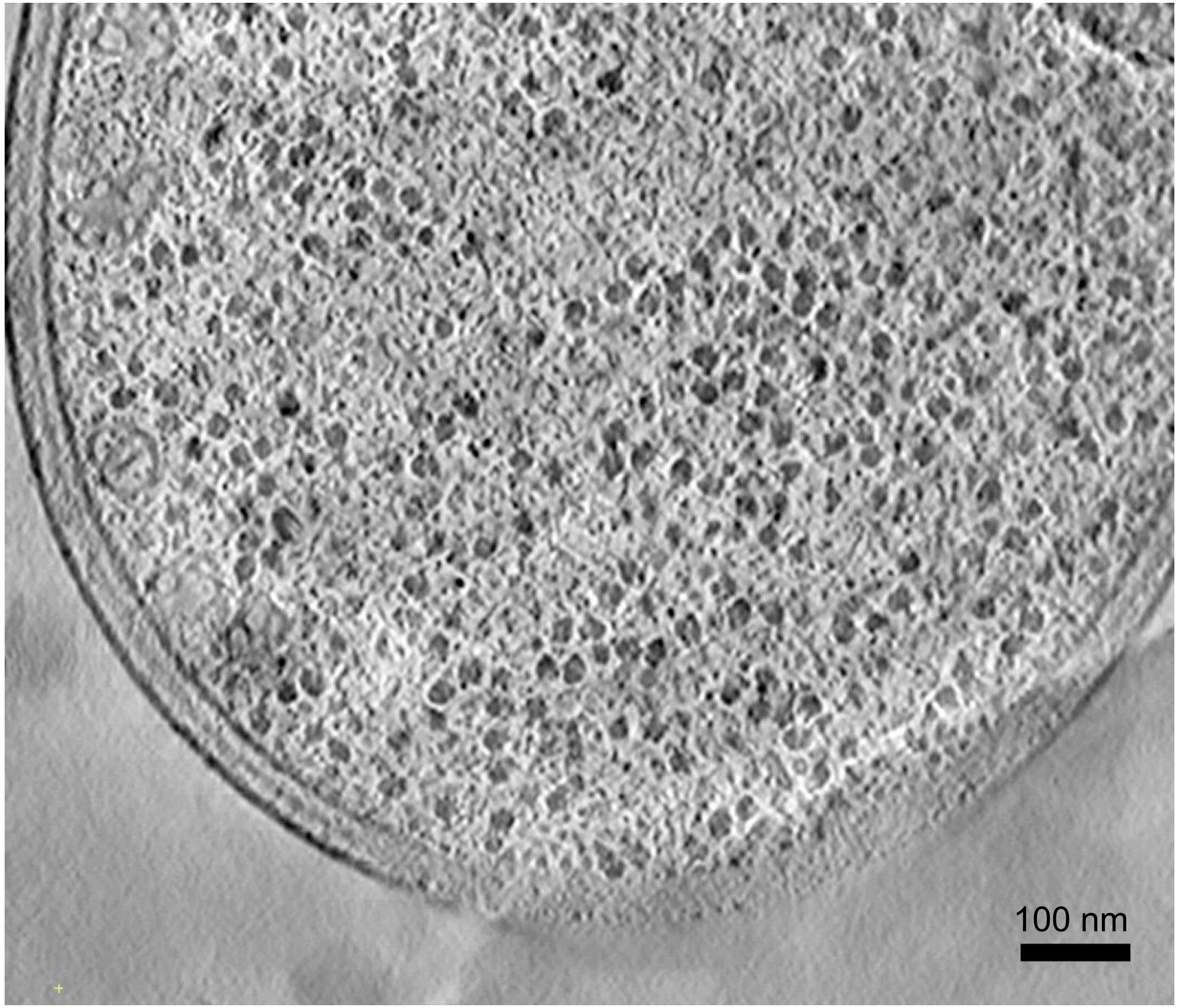
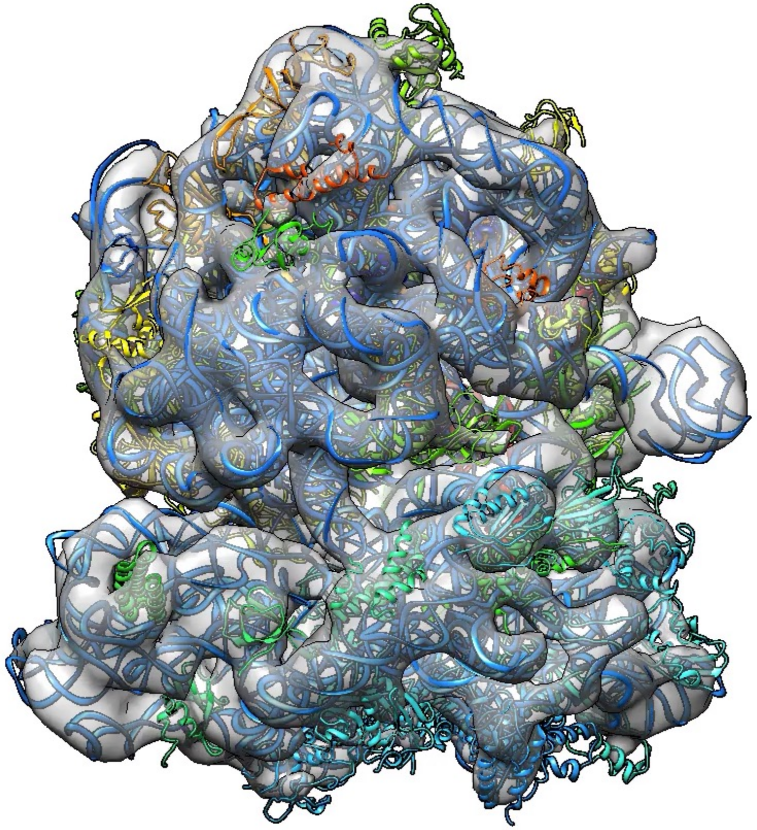
# E. coli visualized using cryo-electron tomography: A “bag of ribosomes”




~70,000 ribosomes per cell



Ribosome is ~20 nm across

PDB 7D80

*Marina Youngblood, Hamid Rahmani,*
***Danielle Grotjahn @ Scripps Research***

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# The Ribosome
is a large machine

- 2.7 Million molecular weight
- 100 by 200 Å
- 2 subunits – Large and Small
- Synthesizes all proteins in the cell

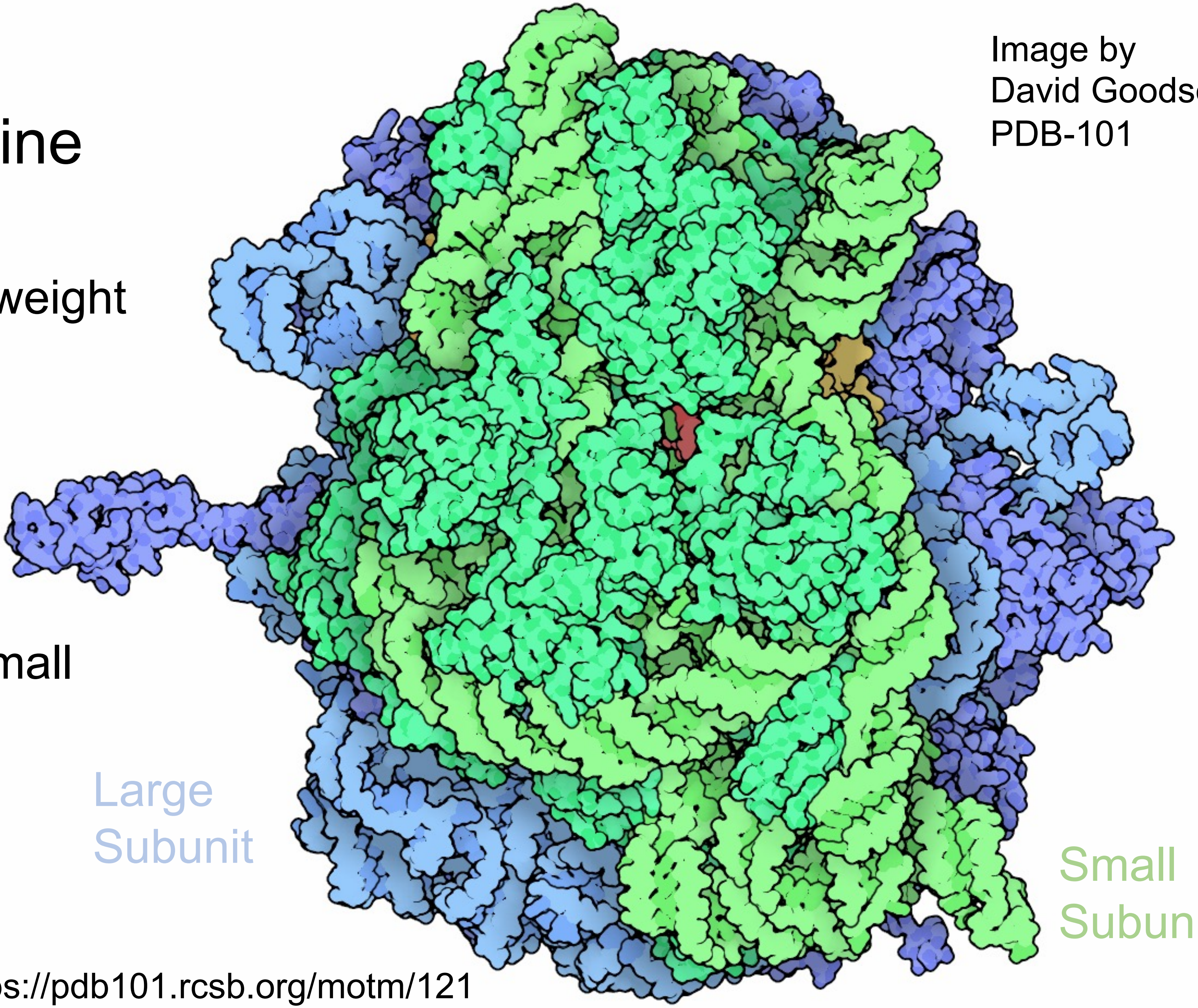



Image by David Goodsell PDB-101

https://pdb101.rcsb.org/motm/121

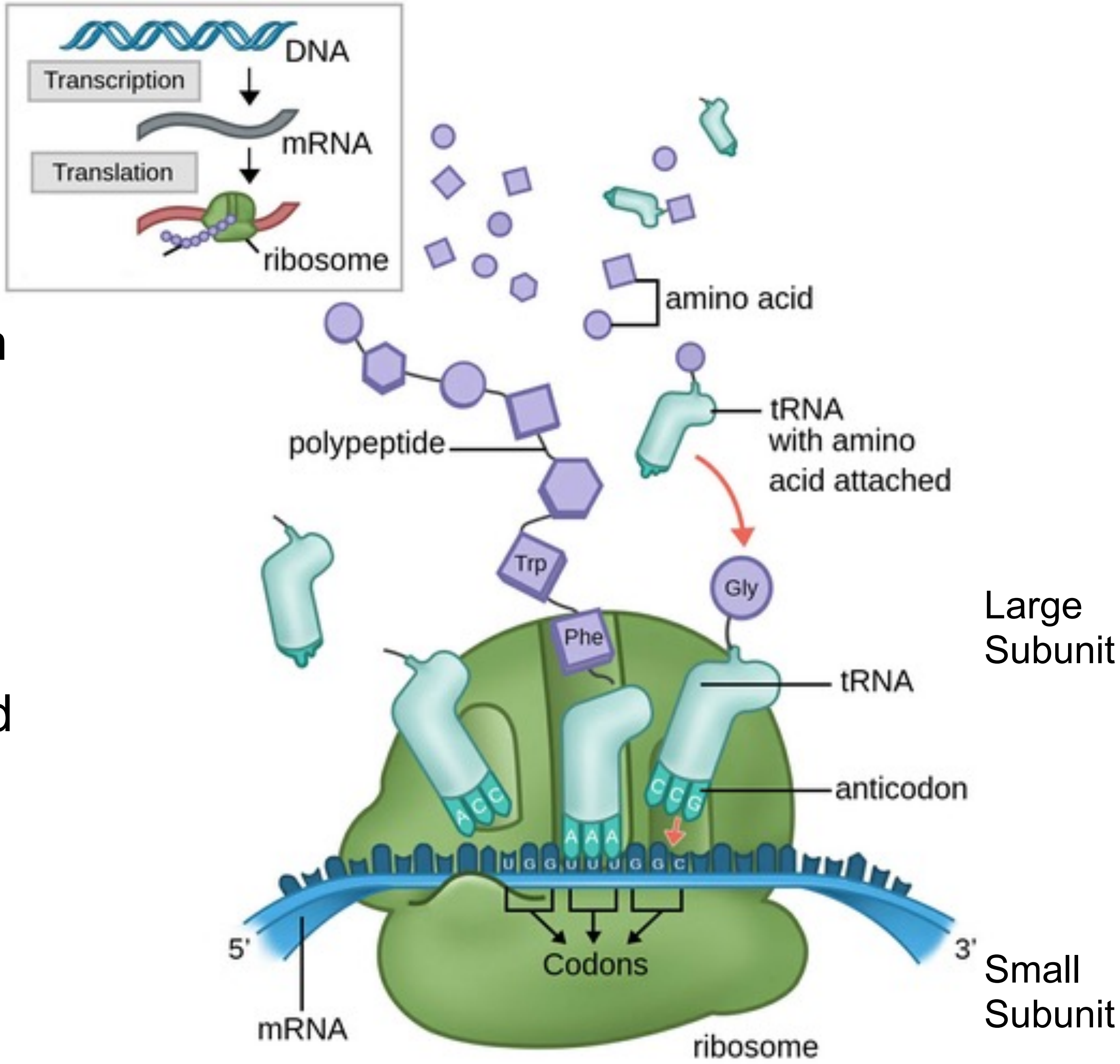
The Ribosome *translates* the messenger RNA into a protein:

- mRNA is threaded between the large and small subunits

- transfer RNAs (tRNA) bind to mRNA codons that specify the amino acid

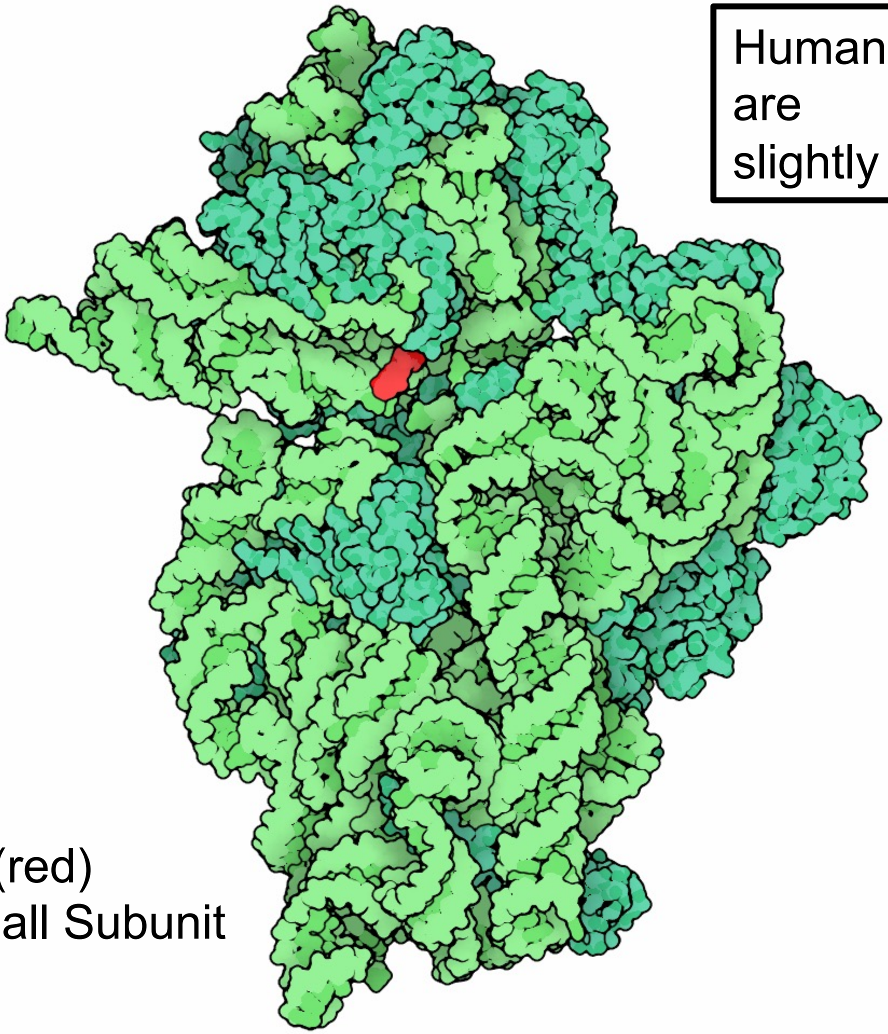
- peptide bond formation is catalyzed

That’s one big enzyme!



# Many powerful antibiotics bind to the ribosome to inhibit translation:

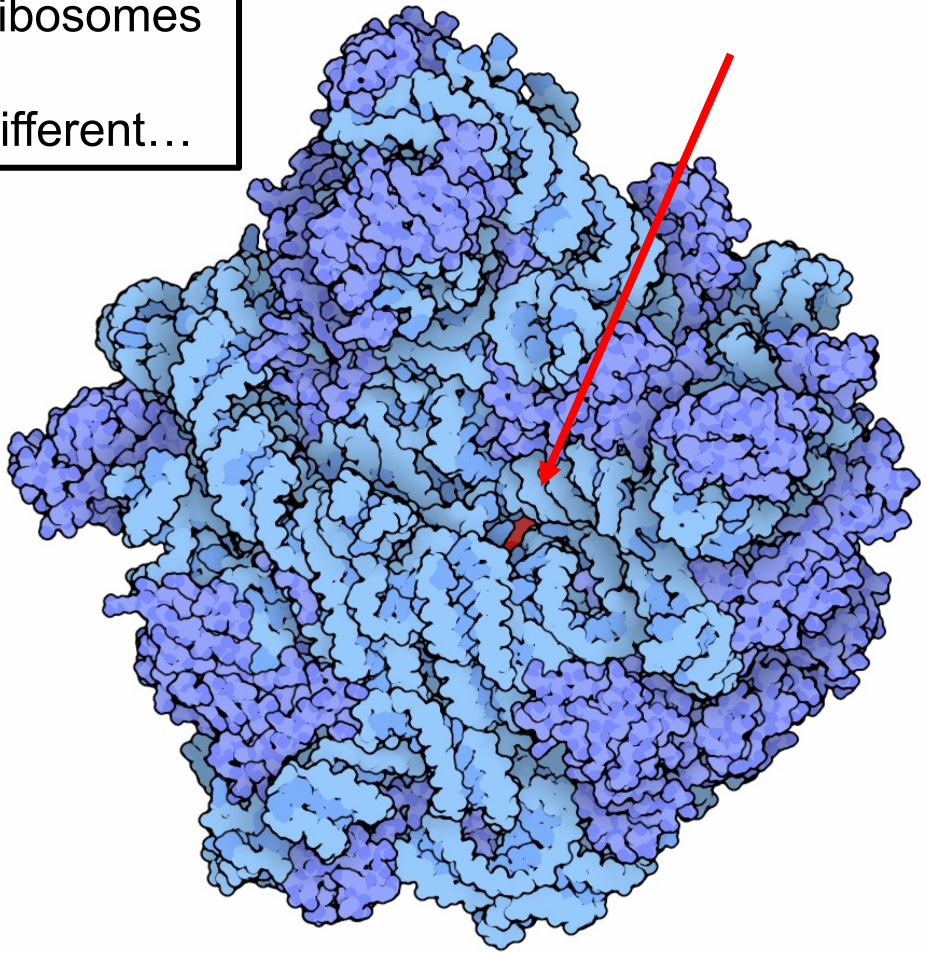
Streptomycin (aminoglycosides)
Tetracycline
Doxycycline
Chloramphenicol
Erythromycin
Azithromycin
Clindamycin
Streptogramins
Linezolid



Tetracycline (red)
Bound to Small Subunit

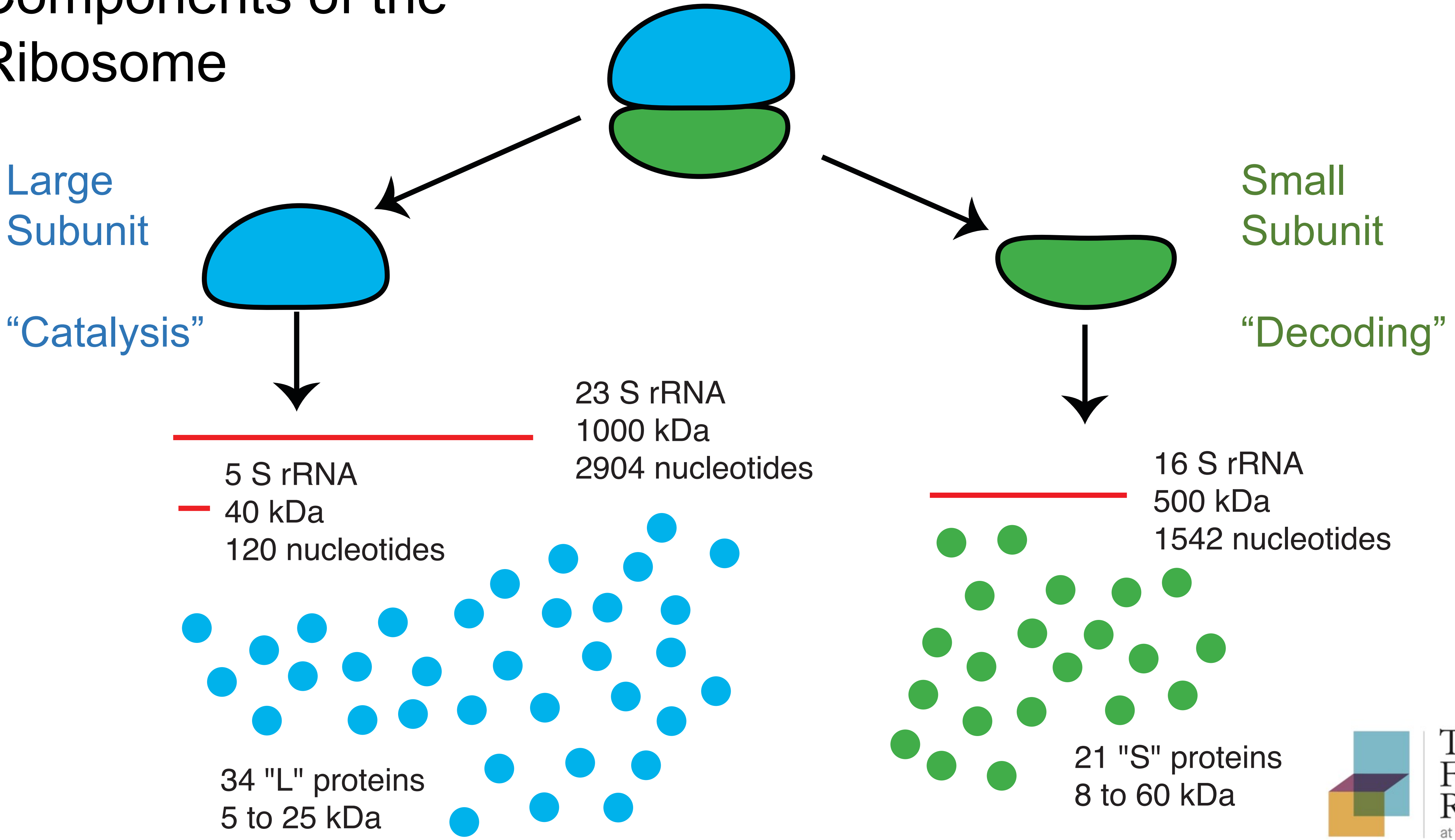
Human ribosomes are slightly different…

Chloramphenicol (red)
Bound to Large Subunit



*David Goodsell again!*

# Components of the Ribosome

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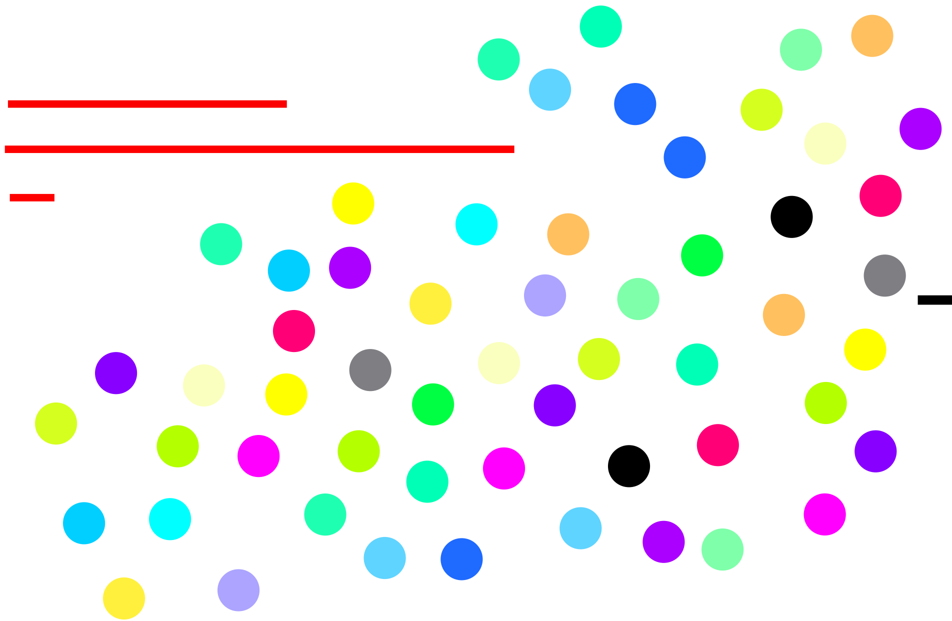
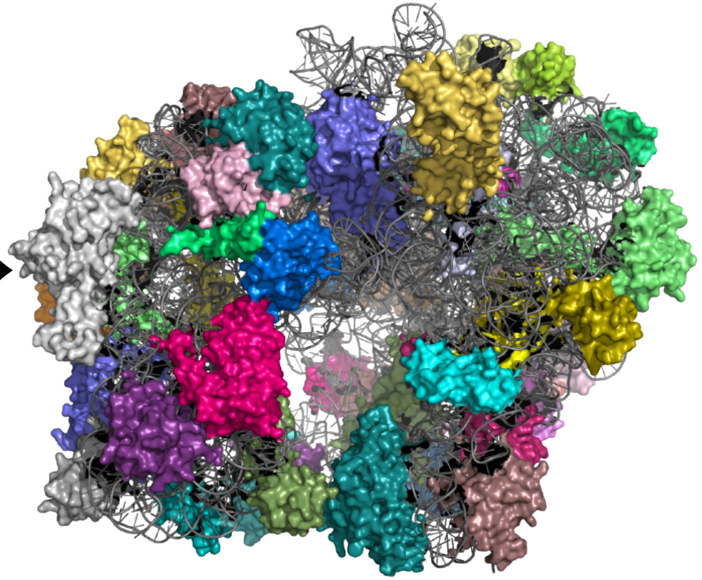
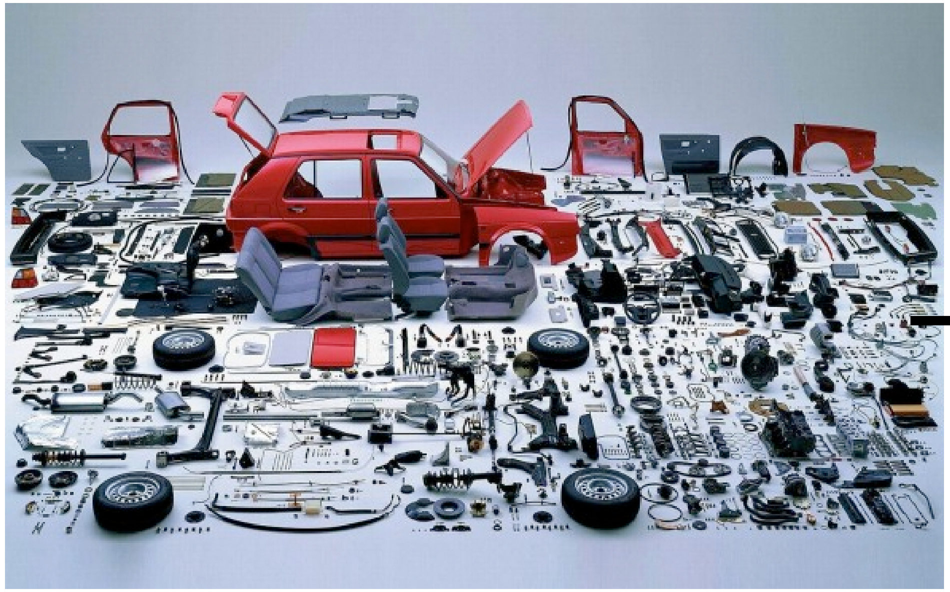
# From Parts List….



# …to Machine





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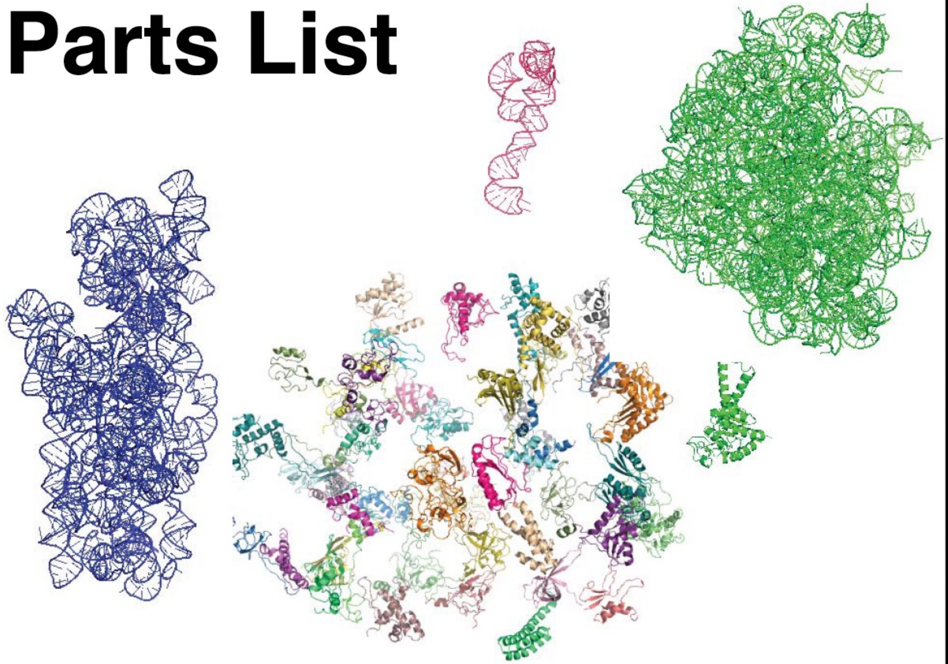
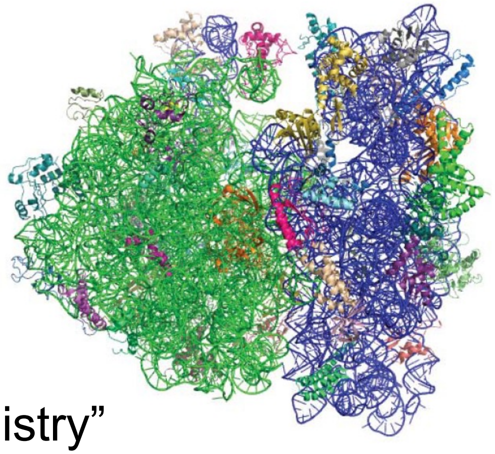
# The Ribosome by IKEA

16S rRNA
20 S-proteins

23S rRNA
5S rRNA
35 L-proteins

**Parts List**

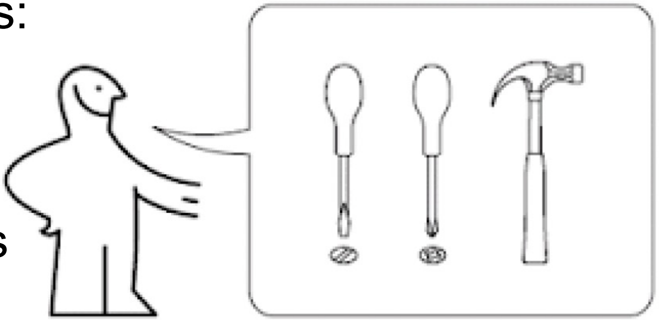





70S ribosome

~ 30 assembly factors:

Chaperones
Helicases
Modification enzymes

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Ribosome Assembly is a fundamentally important biological problem:

- Ribosomes are essential for all cells
- Ribosomes are ~ 1/3 of the dry mass of bacterial cells
- Making ribosomes is *expensive* (lots of ATP required!)
- Ribosome assembly must be efficient for rapid growth
    - bacterial infections
    - cancer cell proliferation
    - cell differentiation

# How are ribosomes assembled in cells?

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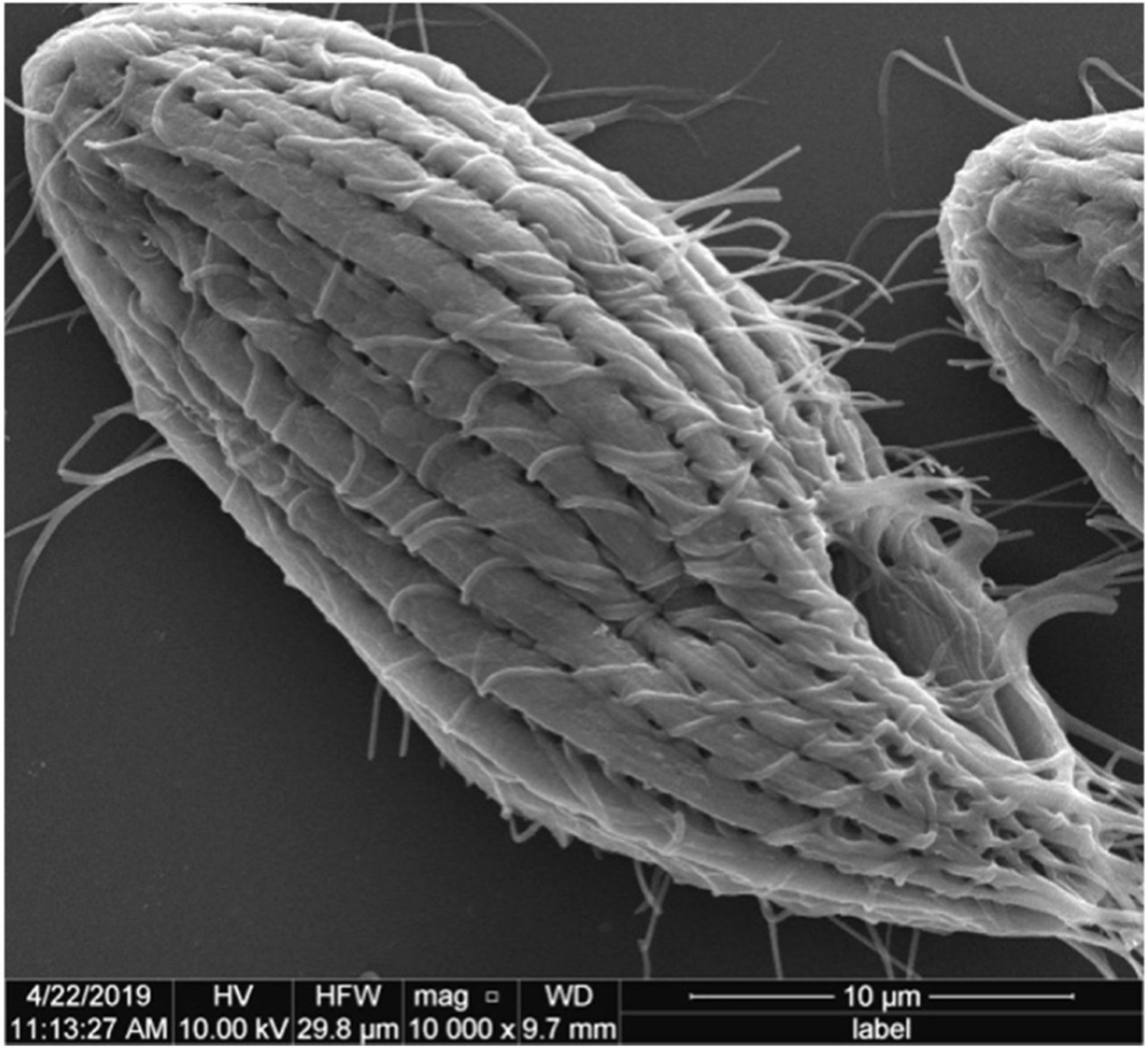
*Tetrahymena thermophila*

- ciliated protozoan
- unicellular
- .02 mm long ~1/1000”
- freshwater lakes, ponds & streams
- grows at 30°C = 86°F

Why study this bug?



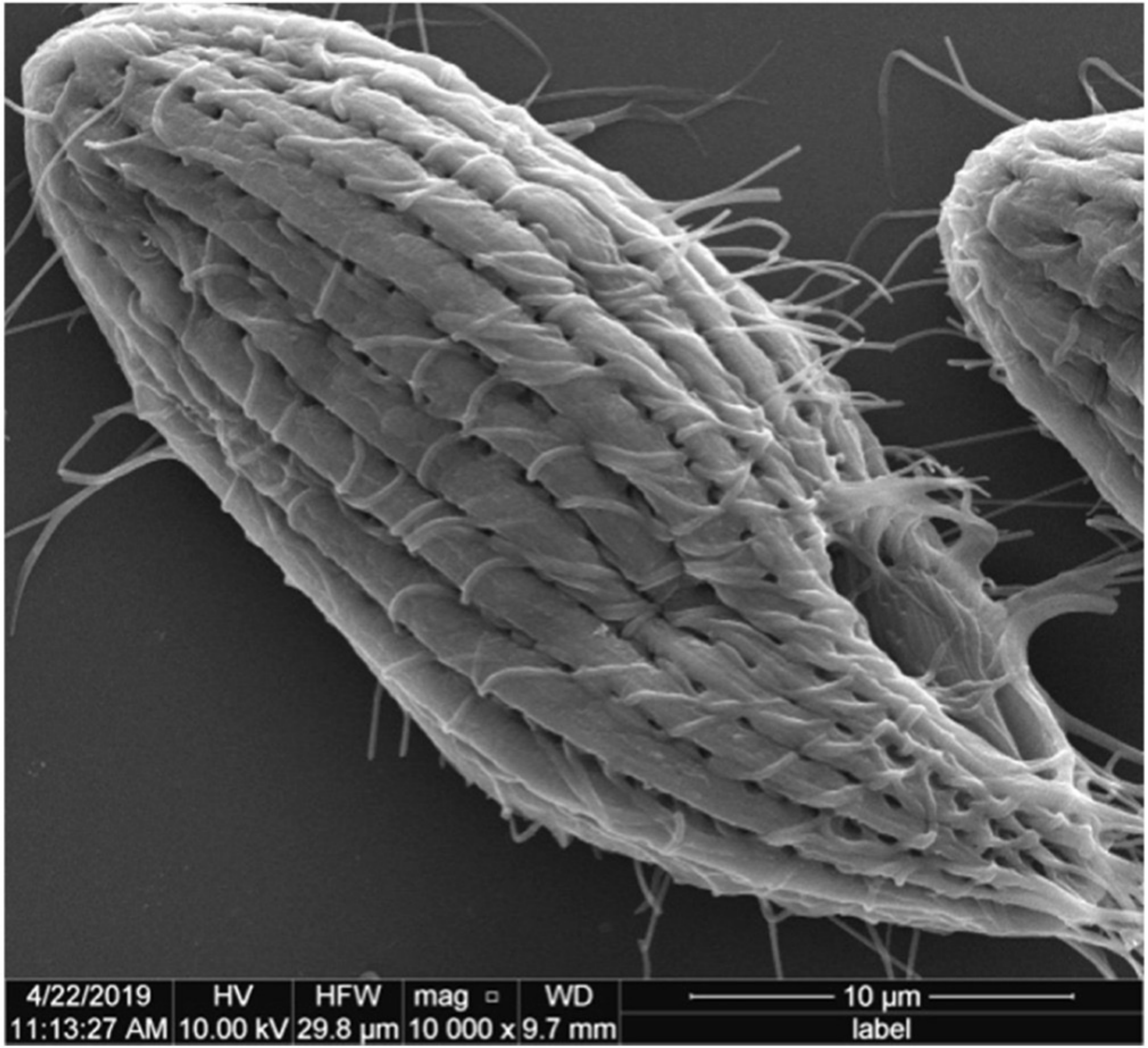
# Tetrahymena has an unusual *genome*

- humans have
 23 very large chromosomes,
 2 copies of each

- Tetrahymena has
 ~200 smaller chromosomes,
 45 copies of each

*Telomeres* are the ends of chromosomes

Humans have 46 telomeres per cell
Tetrahymena has 9000!



# Tetrahymena is an ideal system to study chromosomes
# - basic research on DNA replication was possible, that was difficult in human cells

## The Nobel Prize in Physiology or Medicine 2009

© The Nobel Foundation. Photo: U. Montan

Elizabeth H. Blackburn

Prize share: 1/3

© The Nobel Foundation. Photo: U. Montan

Carol W. Greider

Prize share: 1/3

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Jack W. Szostak

Prize share: 1/3

The Nobel Prize in Physiology or Medicine 2009 was awarded ”for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase”.

Liz Blackburn, former President of the Salk, and Carol Greider did their pioneering work together at Berkeley…

studying Tetrahymena thermophila!

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# One *special* chromosome in Tetrahymena was particularly interesting:

- only one gene – human chromosomes have thousands
- very short – 21,000 nucleotides – humans are millions long
- many copies – 9000 – humans have 2 copies

- Studying the transcription of this gene led to the discovery of "catalytic RNA"

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# The Nobel Prize in Chemistry 1989

Photo from the Nobel Foundation archive.

Sidney Altman

Prize share: 1/2

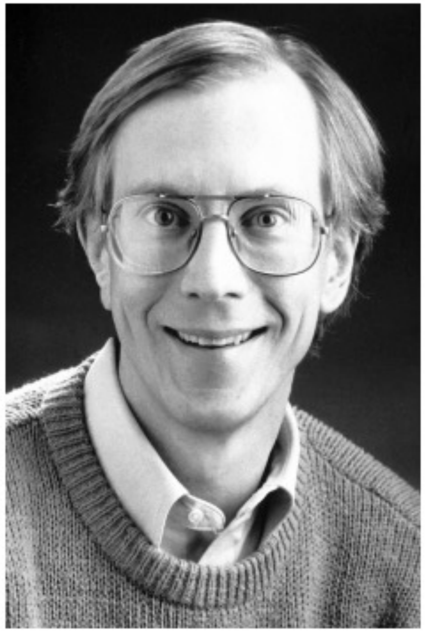

Photo from the Nobel Foundation archive.

Thomas R. Cech

Prize share: 1/2

The Nobel Prize in Chemistry 1989 was awarded “for their discovery of the catalytic properties of RNA”

Tom Cech discovered catalytic RNA while studying the synthesis of RNA from a single chromosome...
in *Tetrahymena thermophila!*

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# Two Connections to today:

JRW

Cech Lab photo, Boulder, CO, October 1989

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# Two Connections to today:

JRW

Cech Lab photo, Boulder, CO, October 1989

AND

The Tetrahymena chromosome that Tom Cech studied encoded the RNA component of…

…The RIBOSOME!!!

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# Why the detour?

- Studying *Tetrahymena* is ”Basic Research” –
  - directed by curiosity and interest
  - not primarily aimed at a disease
  - understanding “How do things Work?!?”
- Two Nobel Prizes resulted that changed science
- Basic Research sets the stage for ALL work on human health
- That’s why we study RIBOSOMES!

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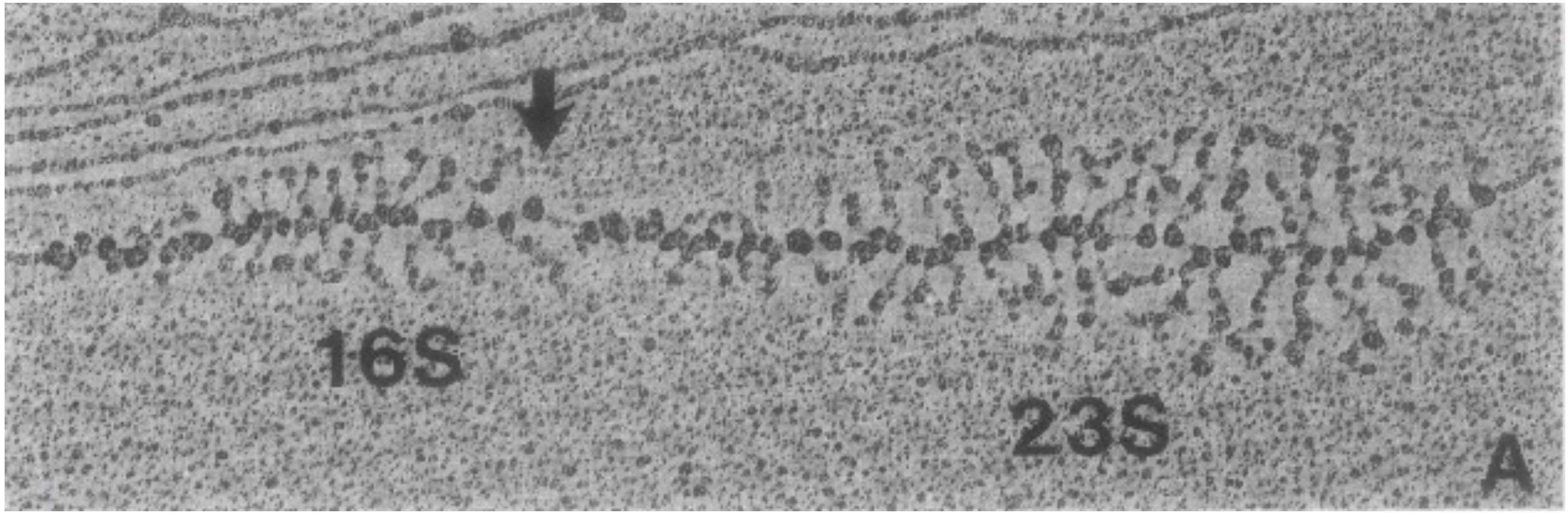
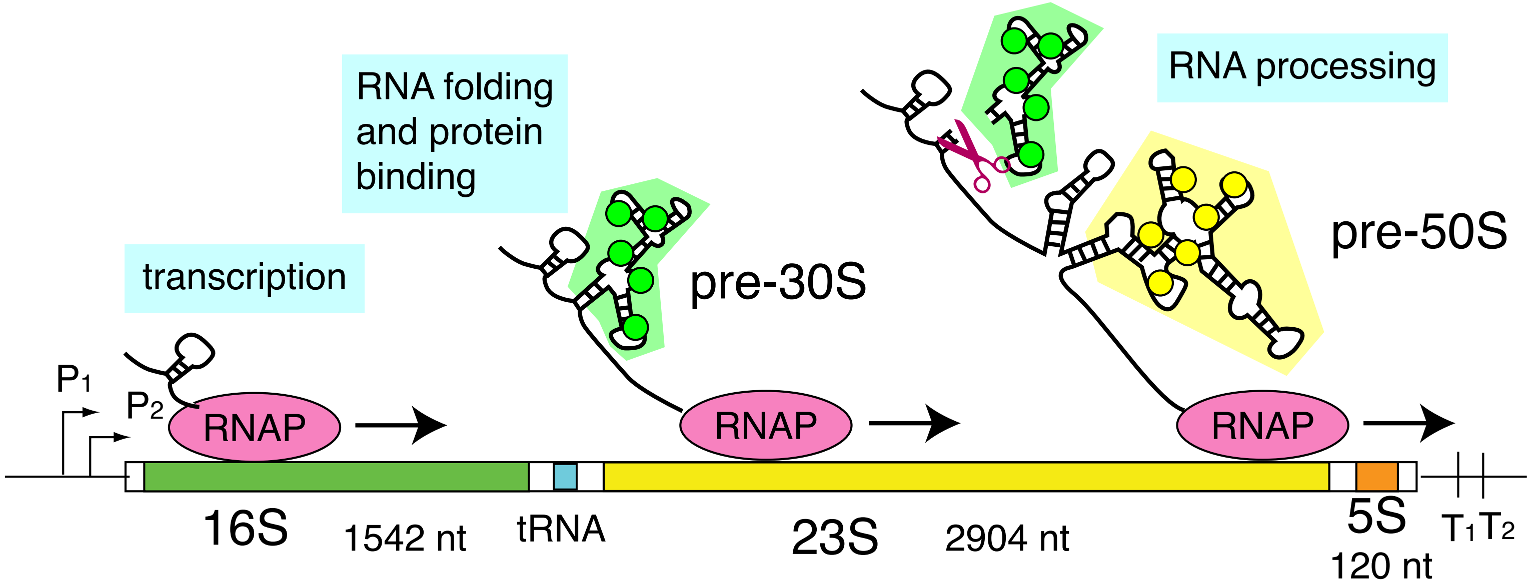
# Electron Micrograph of the ribosome assembly line… "Christmas Tree"




Gotta, Miller & French, *J. Bact.* 173, 6647-9 (1991)

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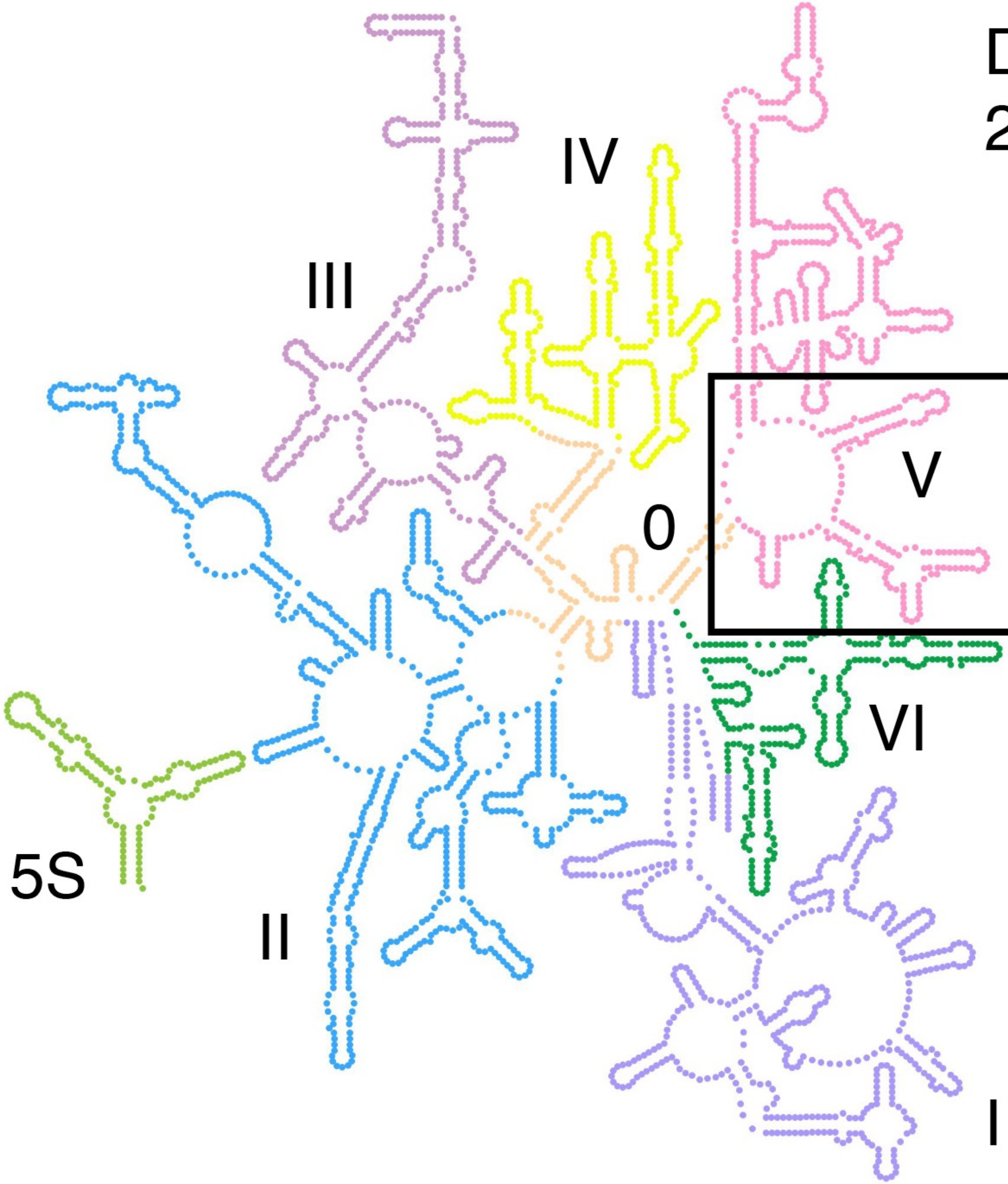
# Domain structure of 23S ribosomal RNA




Domain V harbors the peptidyl transferase active site

The PTC is the most conserved rRNA region, and is presumed to be the ancestral primordial ribosome

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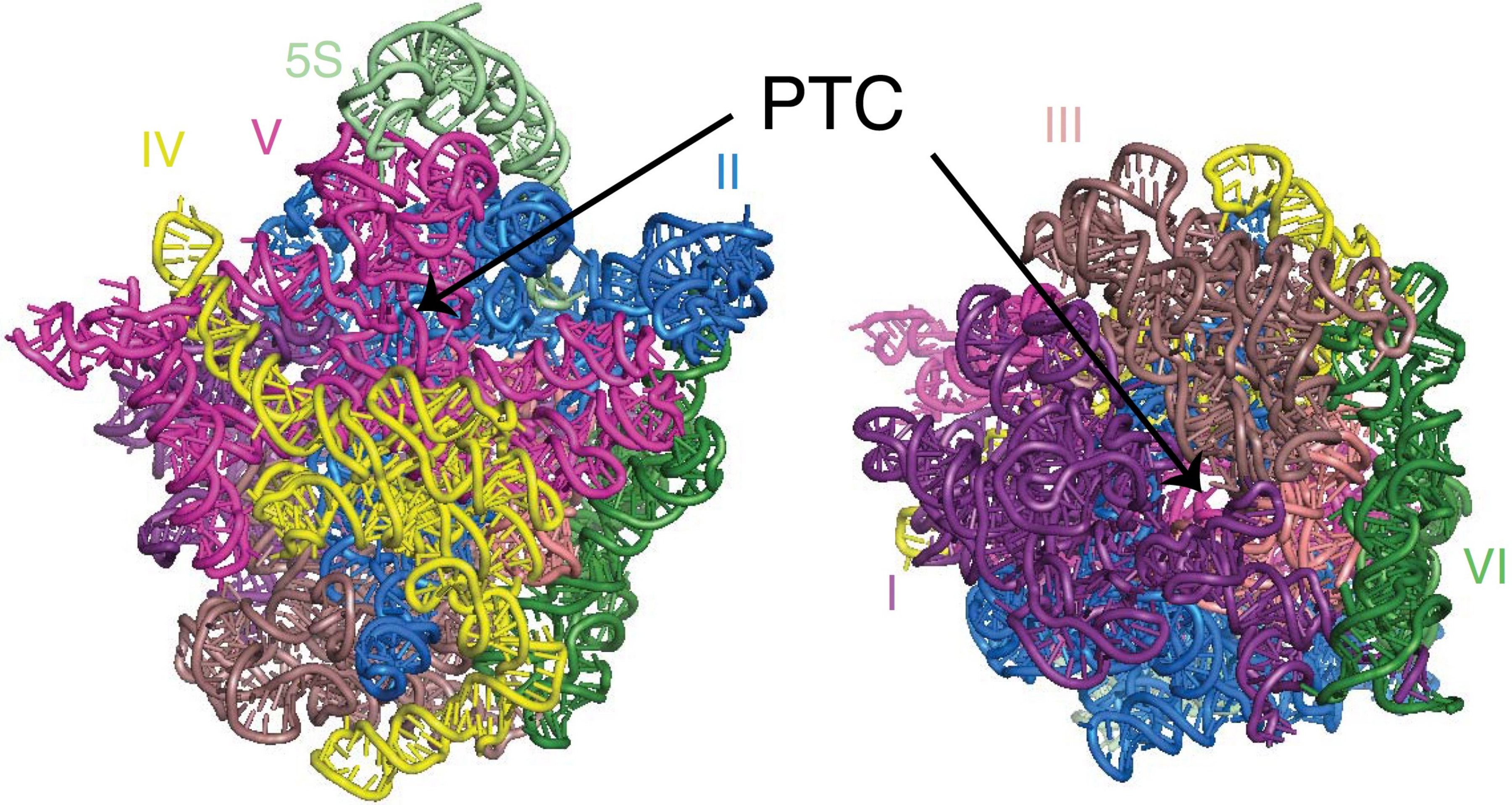
# The domains are highly intertwined

- domains II, IV, V, 5S form the intersubunit interface
- domains I, III, VI form the scaffold




*subunit interface view*
*front*

*exit tunnel view*
*bottom*

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Challenges for looking at ribosome assembly in cells:

- it is *fast*…2 minutes in *E. coli*
- intermediates are transient
- assembly intermediates are not abundant
- hard to find them in cells for a detailed look

Our solution:

- We *perturb* ribosome assembly in such a way that intermediates accumulate for us to observe

- We *limited* the production of an essential ribosomal protein
- protein L17

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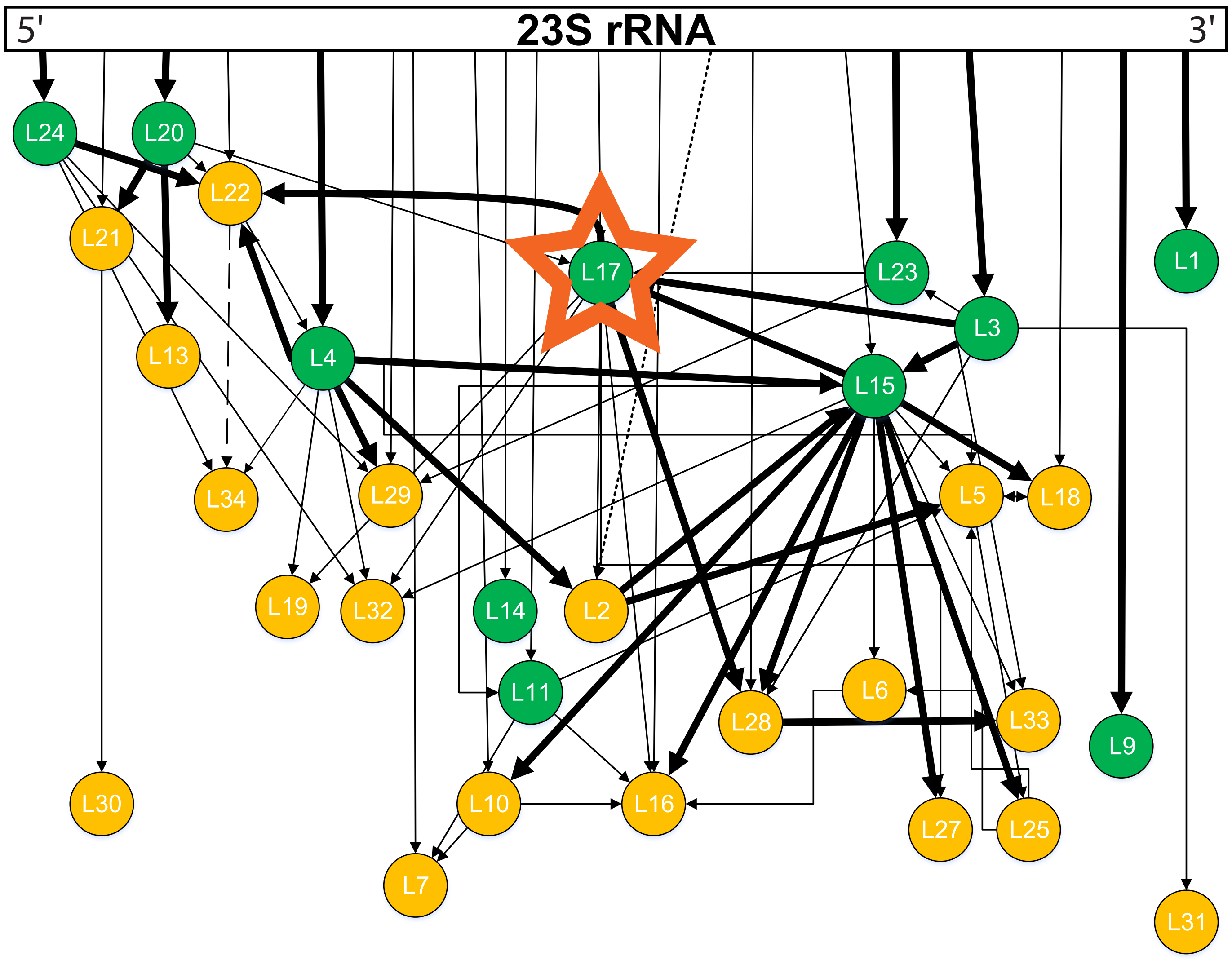
# Protein L17 binds “early”.  If we limit L17, assembly should be impaired




“Nierhaus” Assembly Map

- order of addition of the 35 “L” proteins
- *in vitro* assembly
- heroic work from the 1970s!
- The Rosetta stone for ribosome assembly

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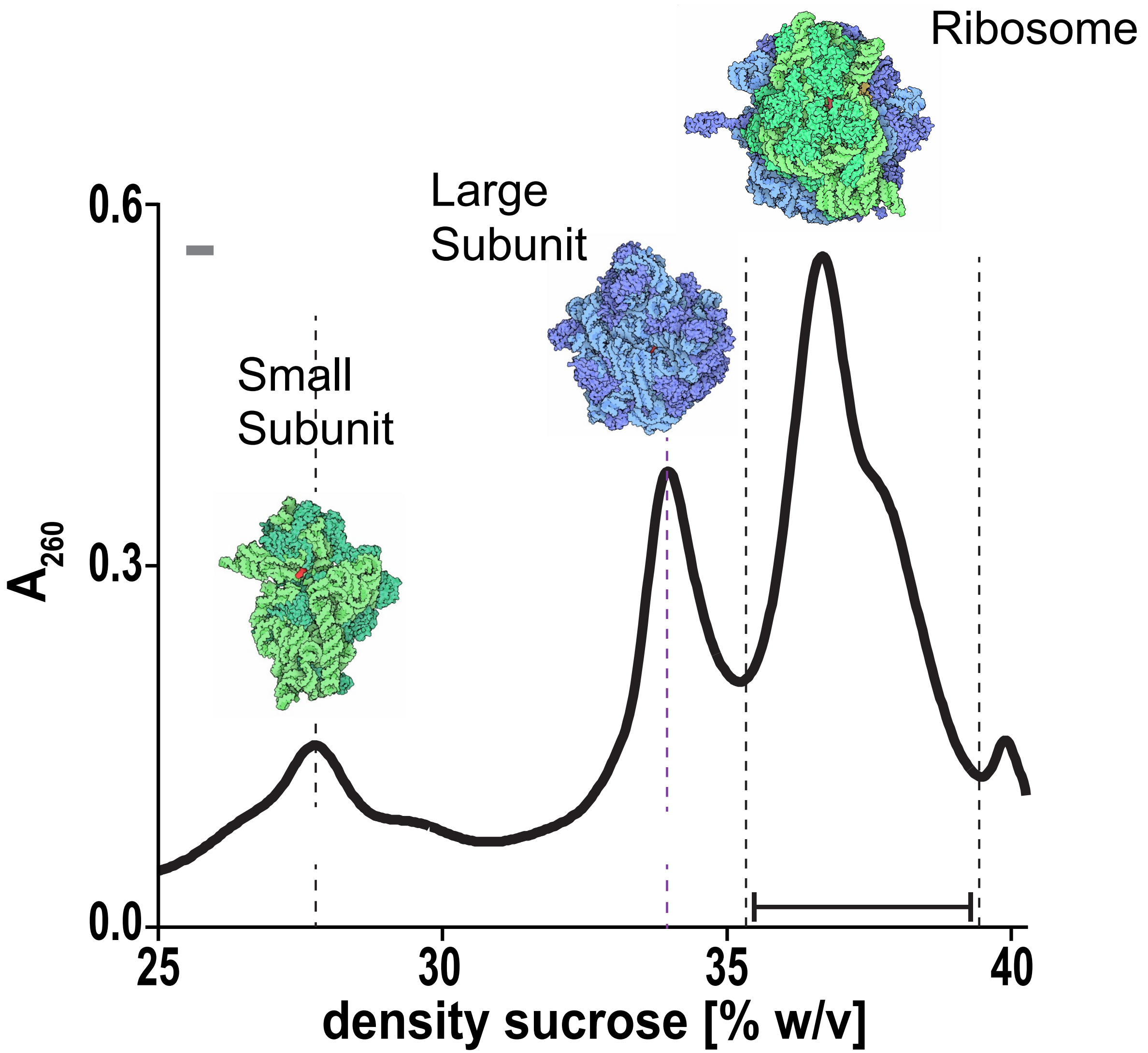
# Ribosome Profile from normal cells




- We observe ribosomes using an ultracentrifuge
- Particles are separated by mass
- RNA absorbs UV light

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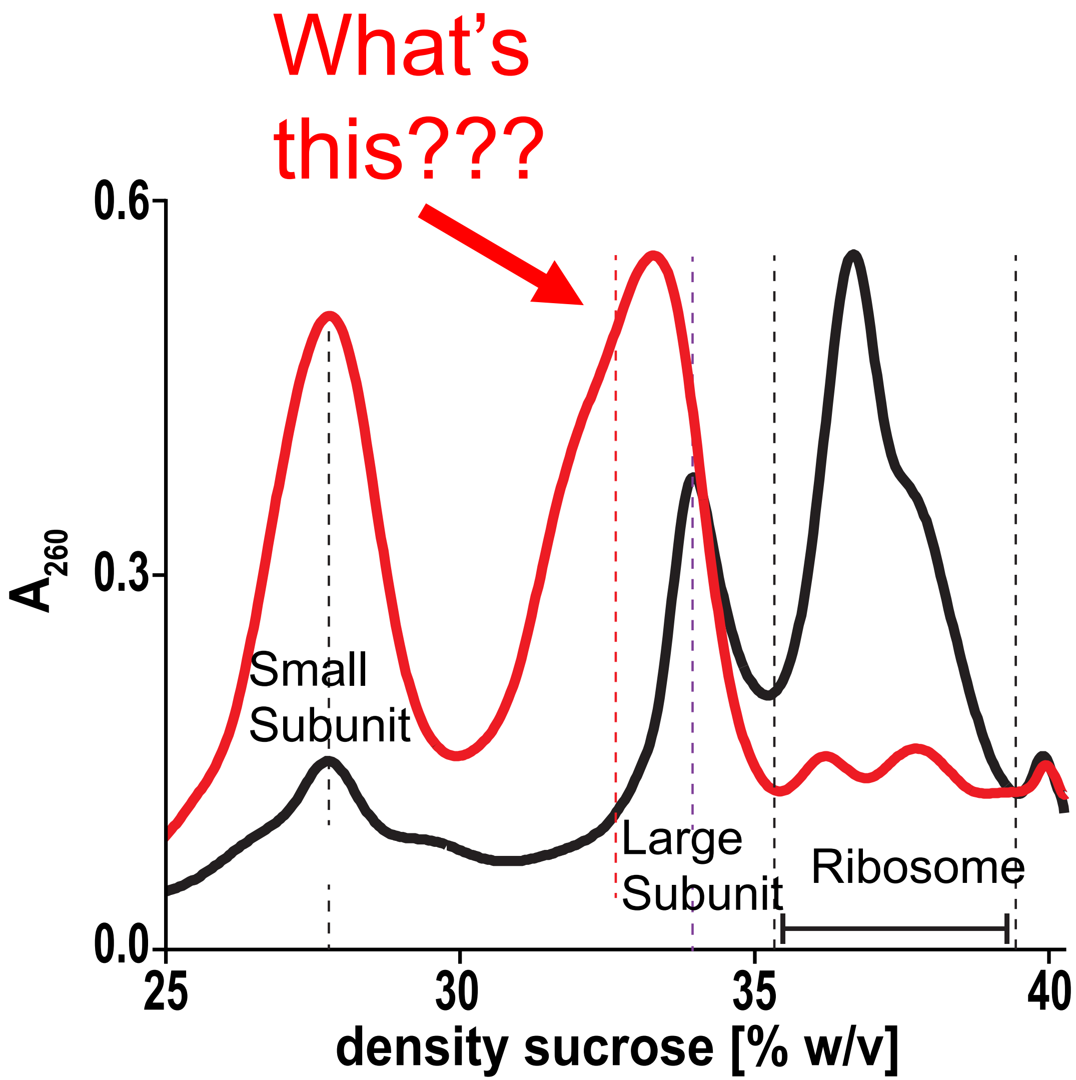
# Ribosome Profile from *perturbed* cells




- When we limit production of L17…

…a new kind of particle accumulates

- How can we look a it?

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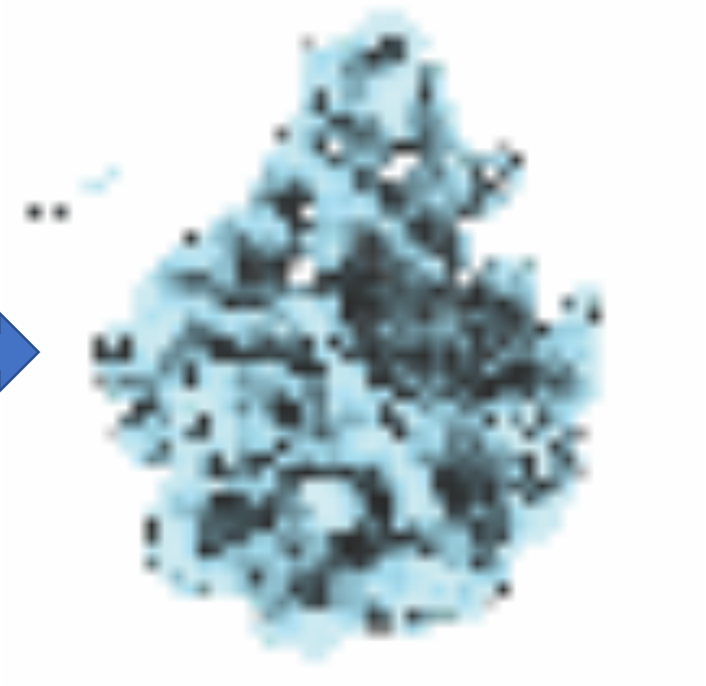
# Cryo-Electron Microscopy: visualizing molecular structures

Titan Krios
Electron Microscope

2-Dimensional
Projection Images



Electron Density Map

Collaboration with Dmitry Lyumkis @ Salk

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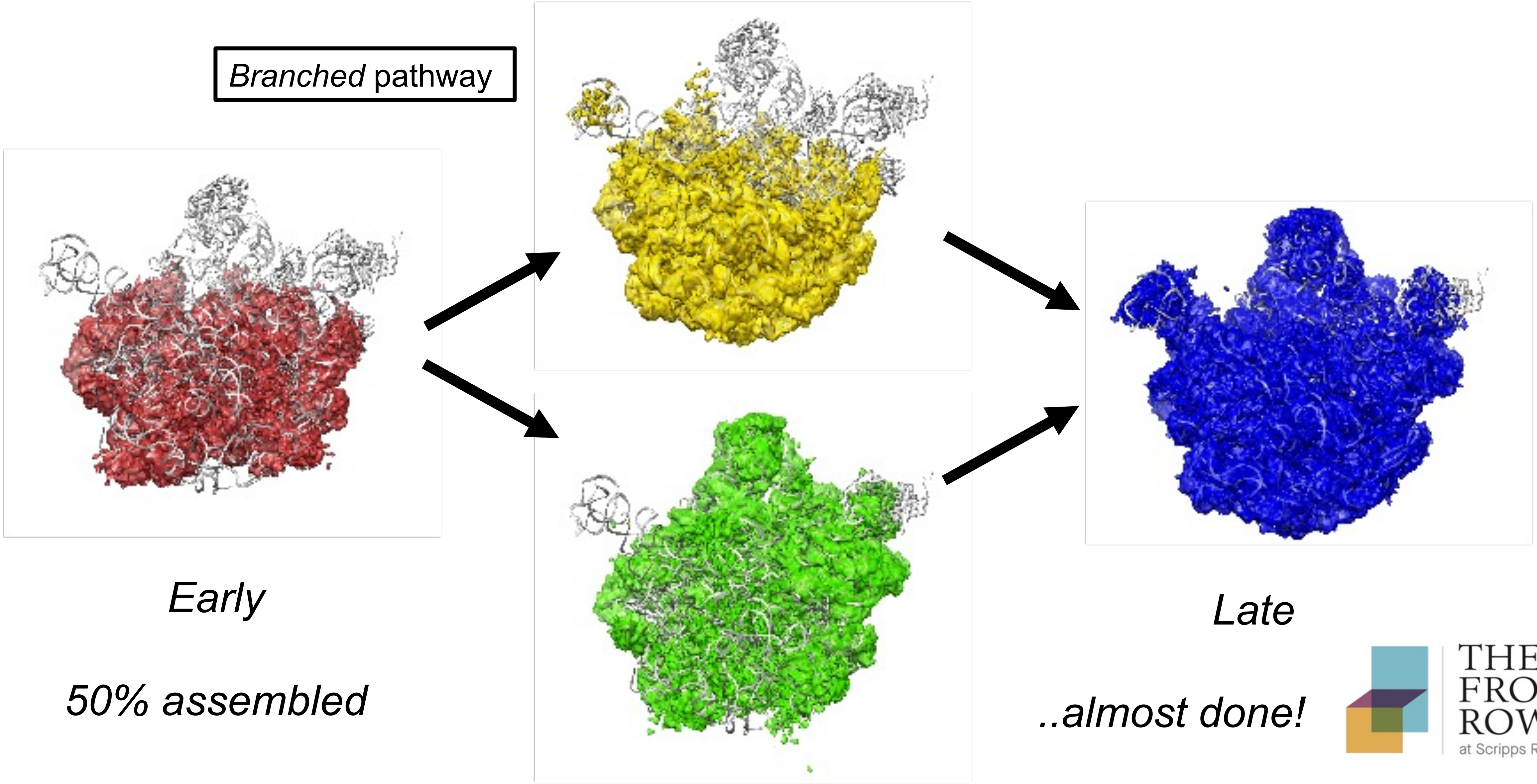
# Partially assembled ribosomes missing L17

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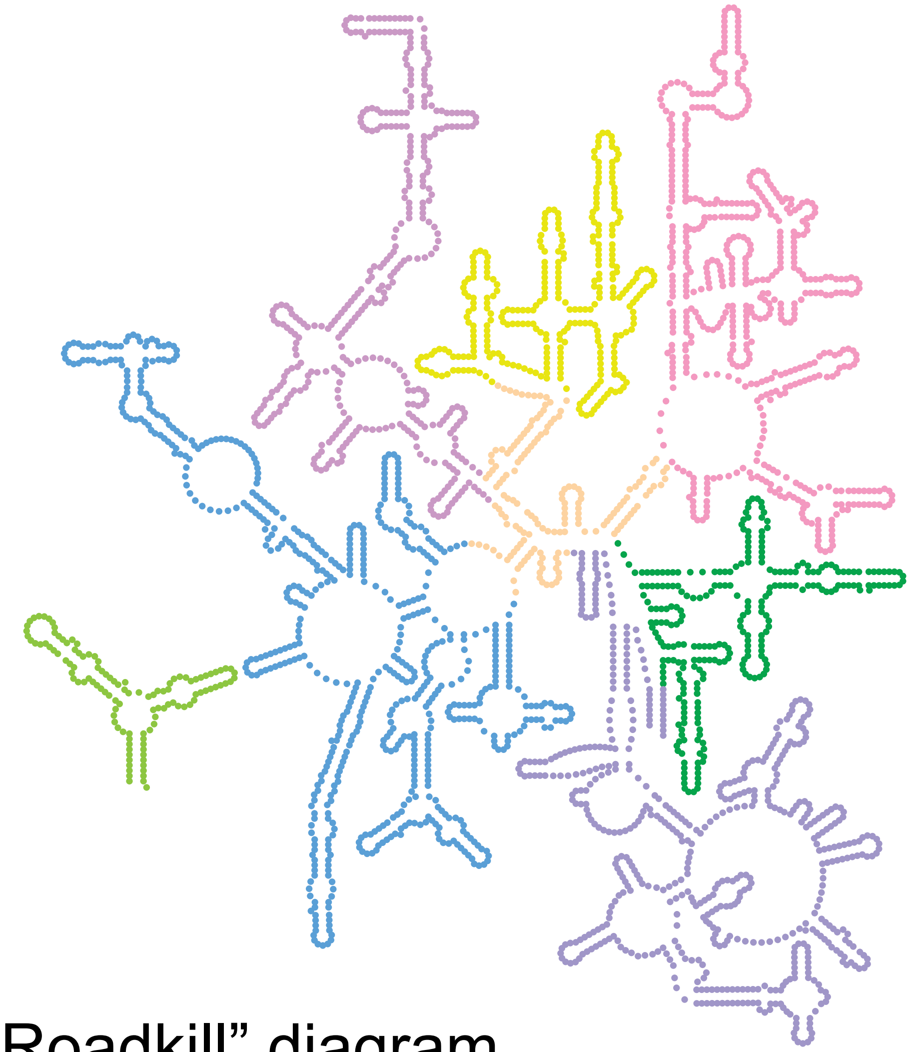
# Domain structure for RNA



“Roadkill” diagram

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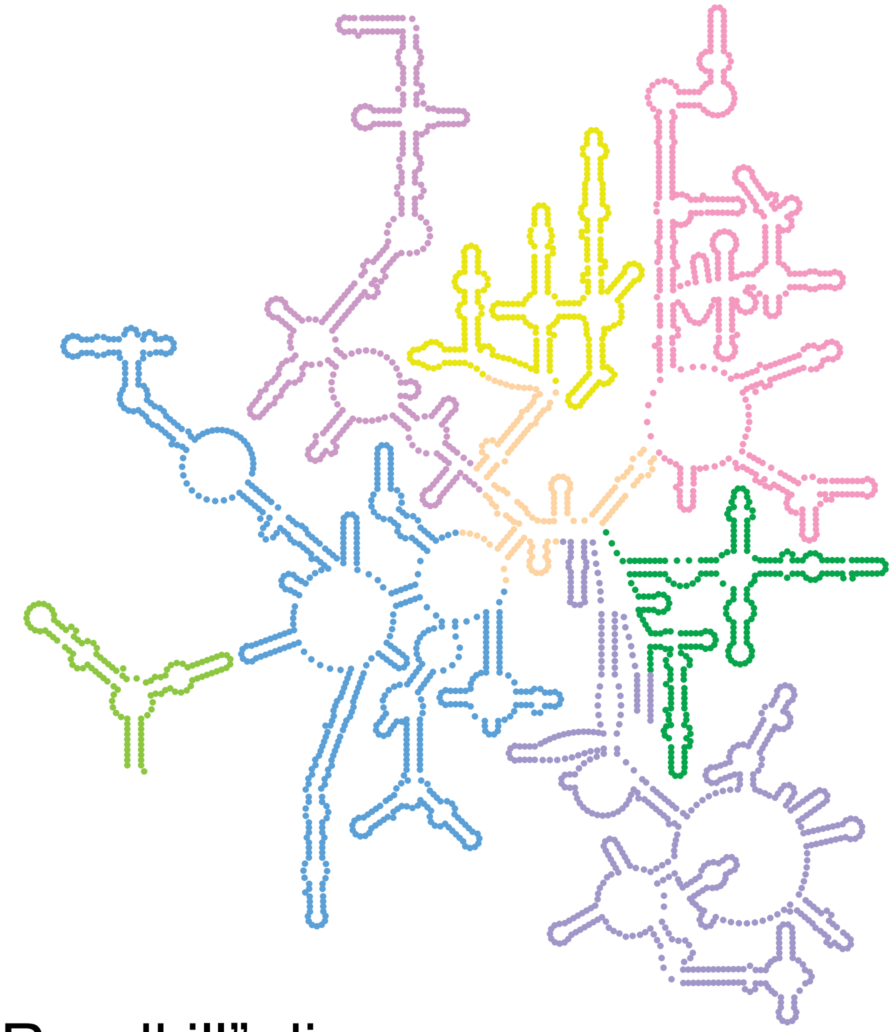
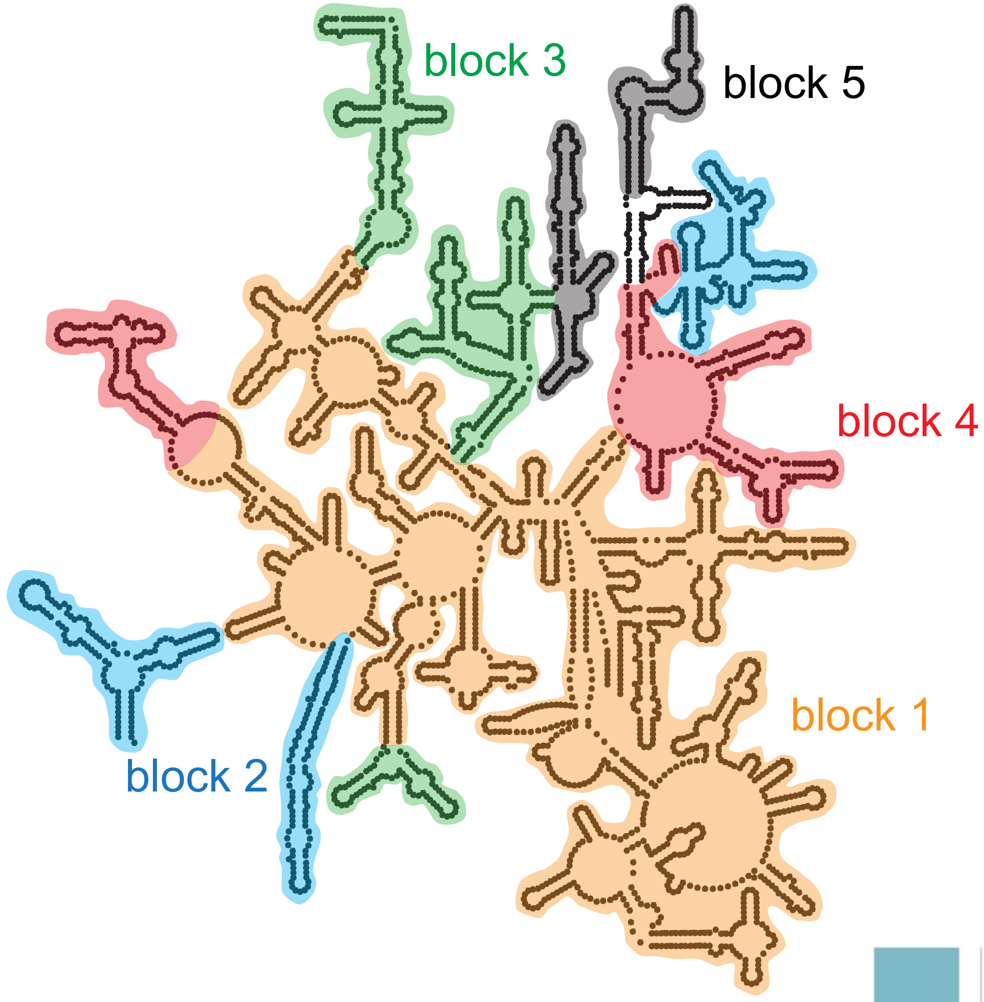
# Cryo-EM reveals order of assembly in blocks



“Roadkill” diagram

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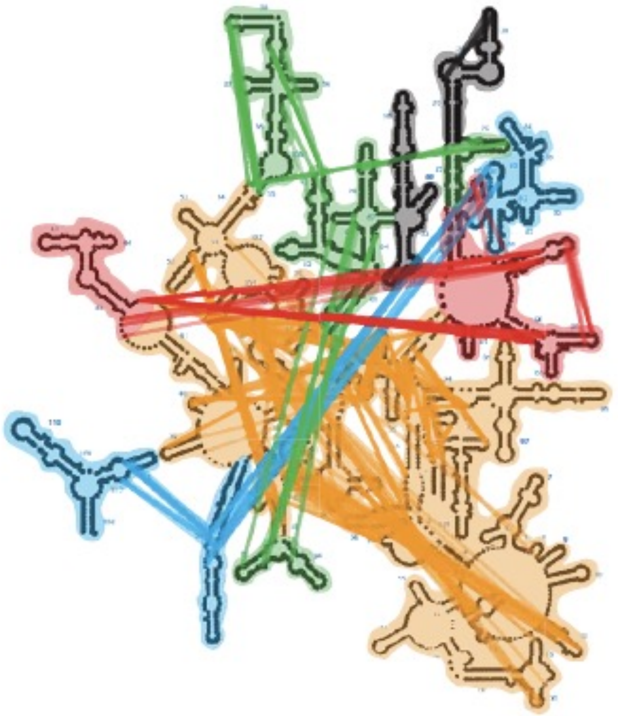
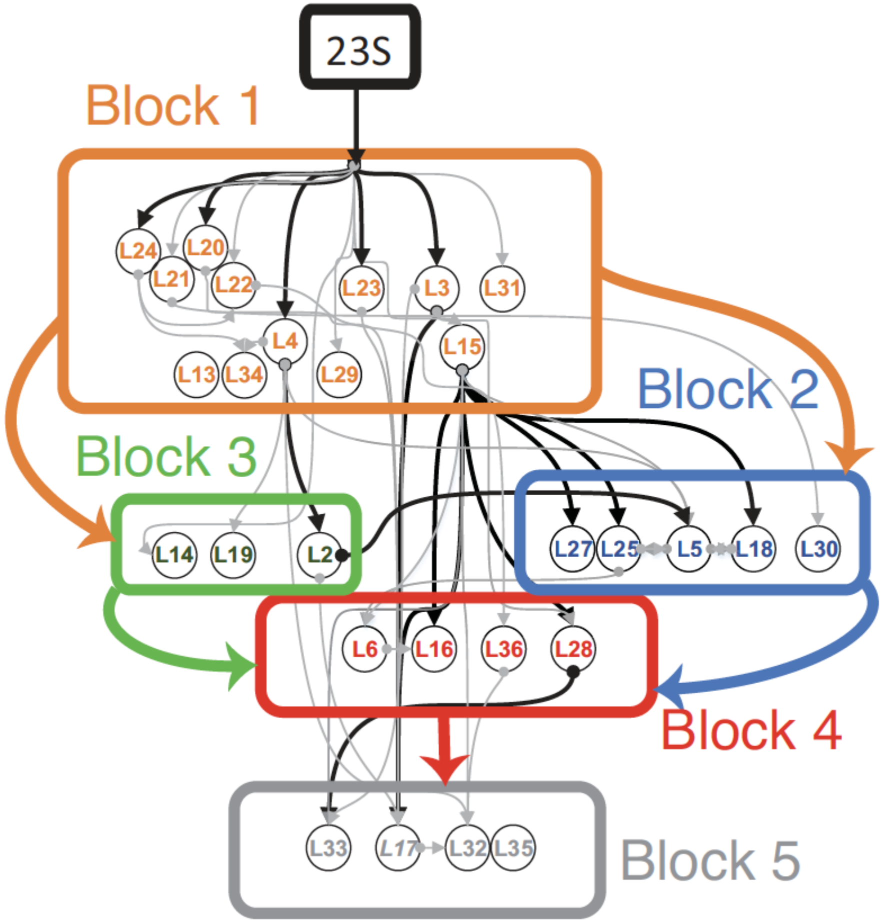
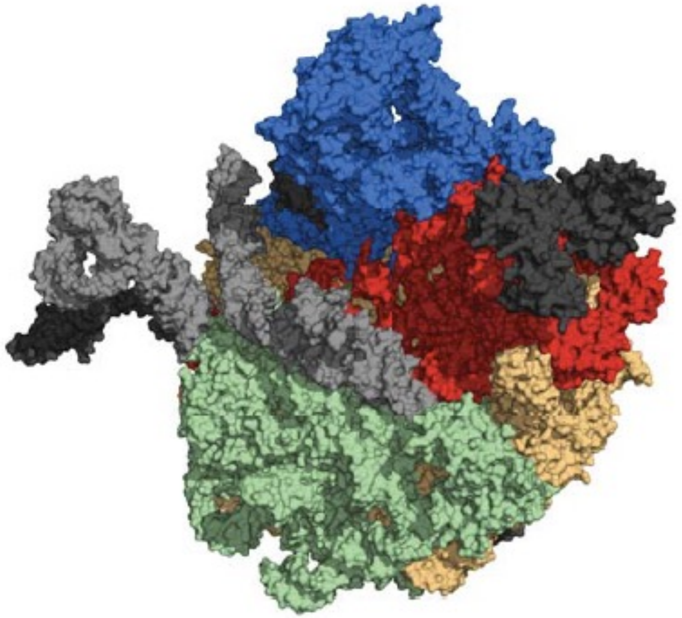
# Three views of our first attempt at an assembly pathway



RNA structure
Roadkill view



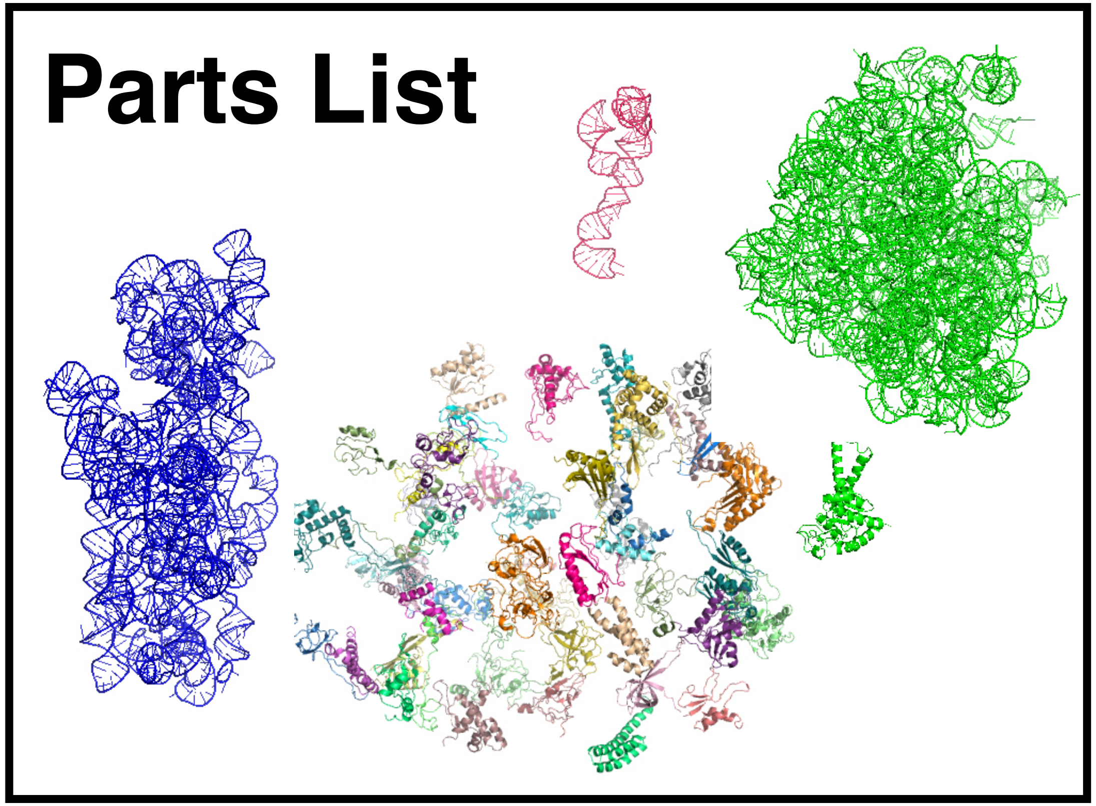

Protein binding
"Nierhaus" view



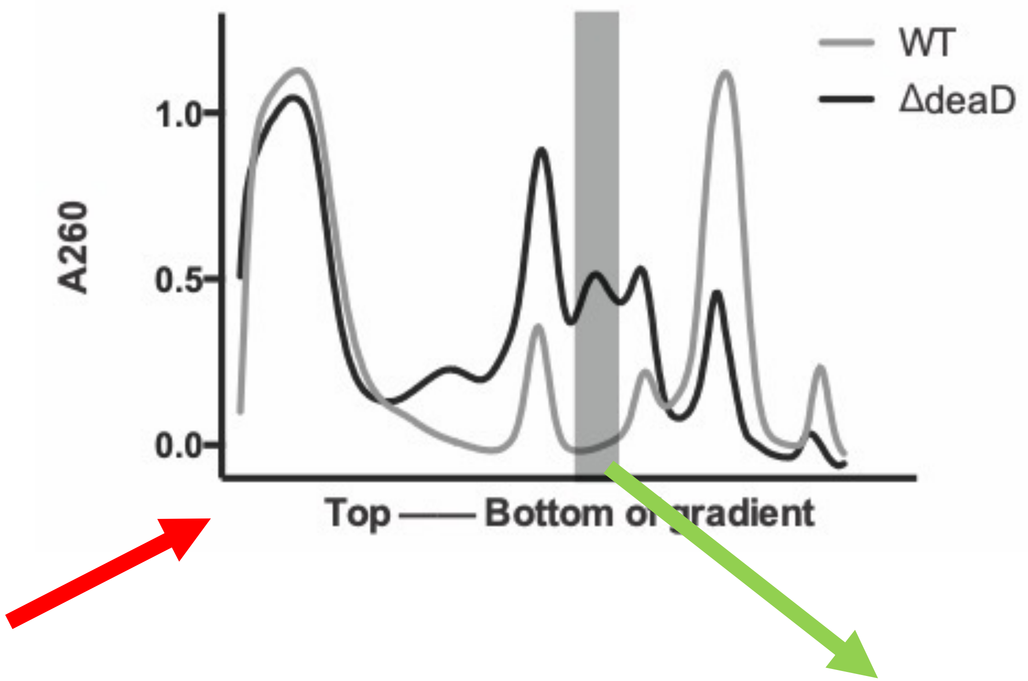
Electron density
Structure view

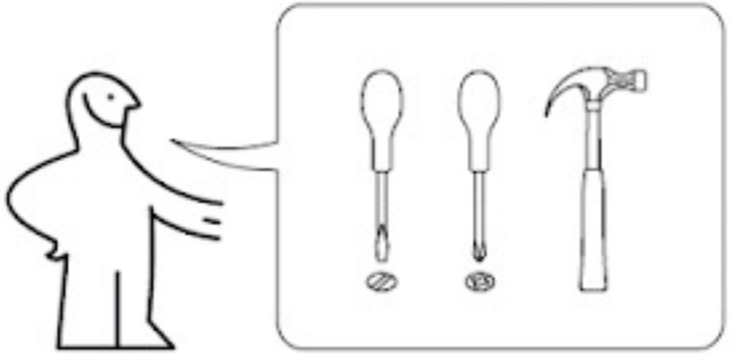
Davis, Tan, Carragher, Potter, Lyumkis, Williamson, *Cell 2016*

# More than 35 cellular proteins assist with assembly




Genetic Knockout of helicase DeaD
Leads to accumulation of intermediates



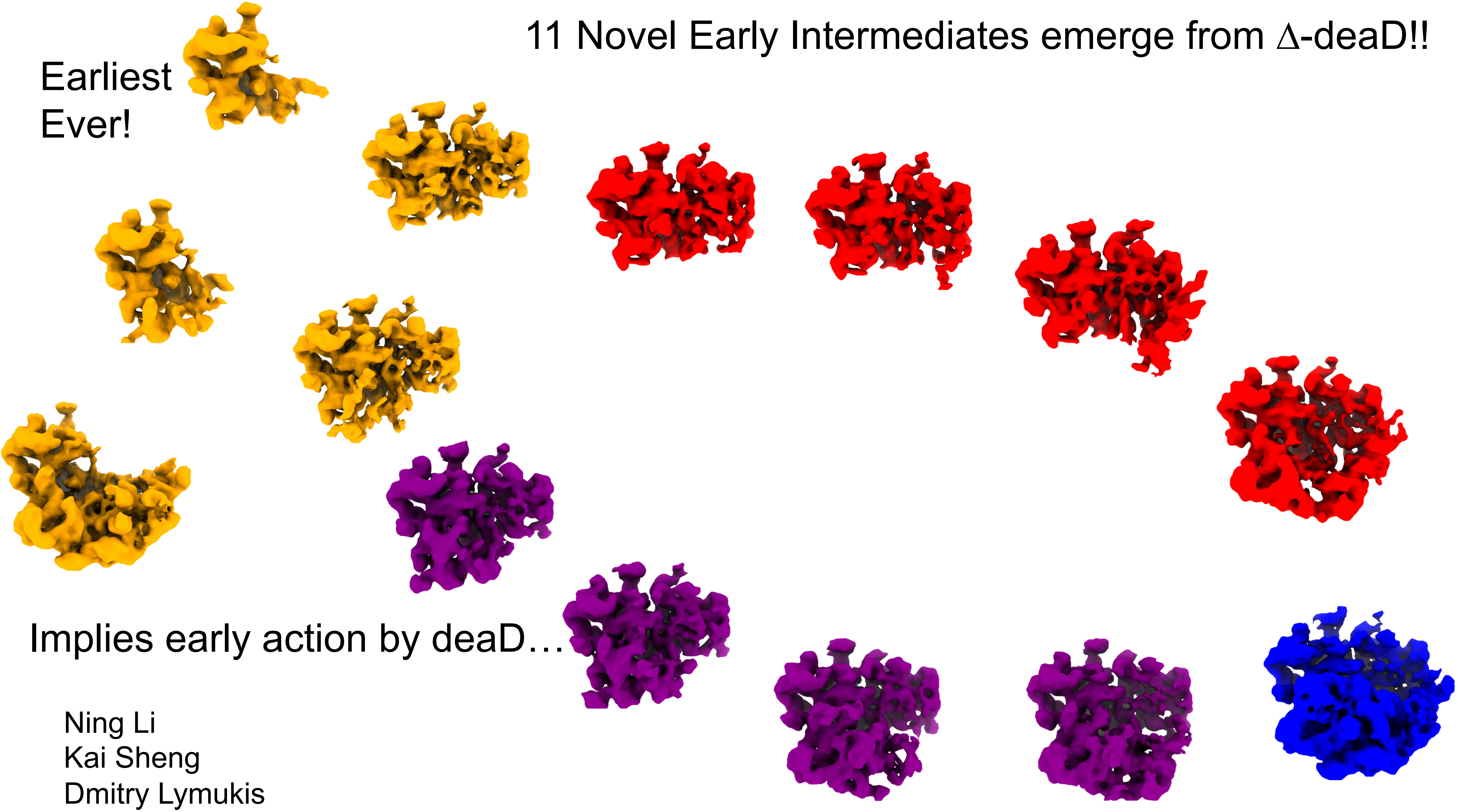
Helper proteins:

*Helicases – ATPases*
GTPases
Chaperones
Methyltransferases
Pseudouridylation

Cryo-EM!!

## 11 Novel Early Intermediates emerge from Δ-deaD!!

Earliest Ever!



Implies early action by deaD…

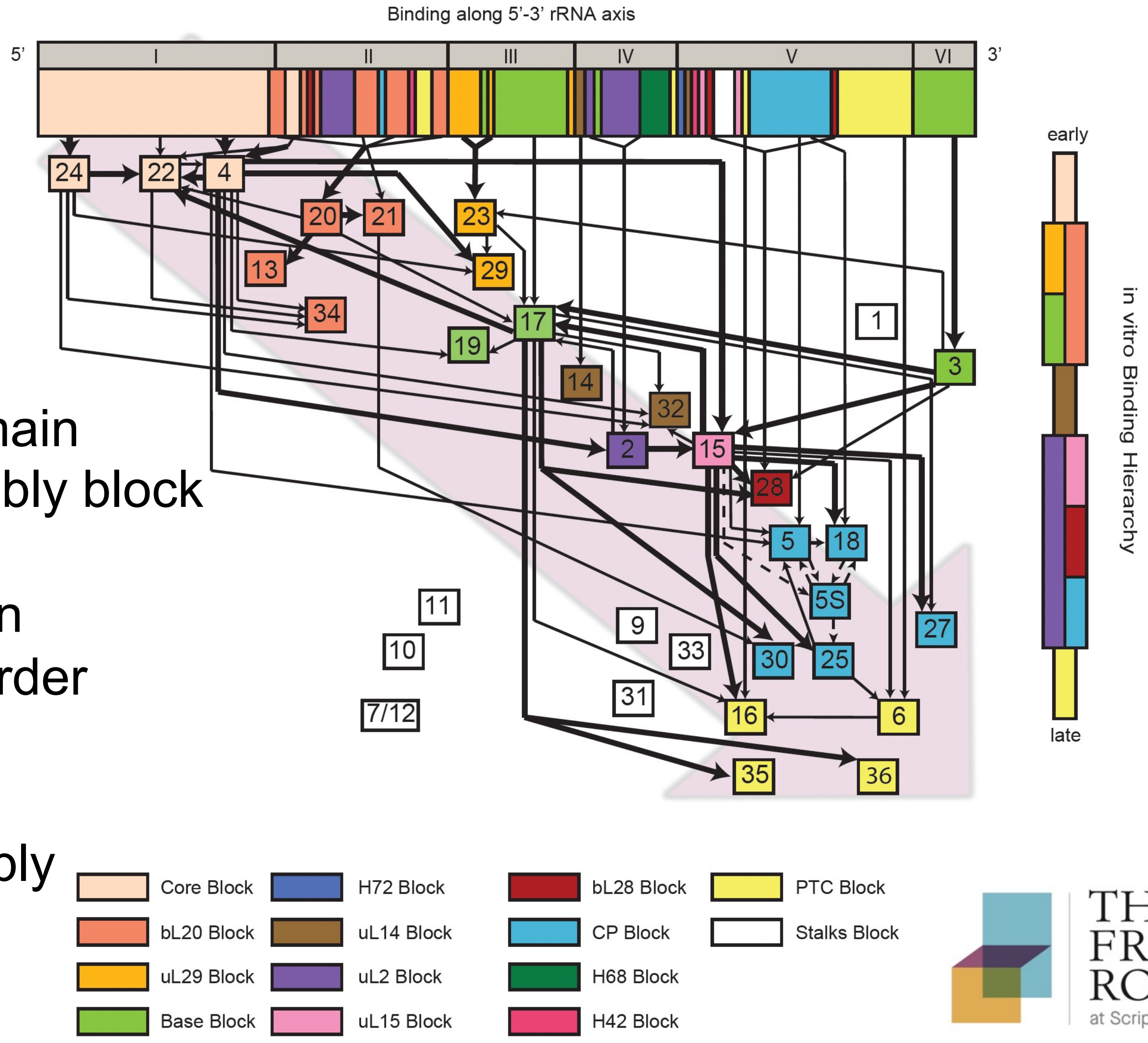
Ning Li
Kai Sheng
Dmitry Lymukis

# Detailed order of events is emerging

- organized horizontally by domain
- organized vertically by assembly block

- 5' to 3' assembly is reflected in *in* vitro protein binding order

- consistent with cotranscriptional assembly

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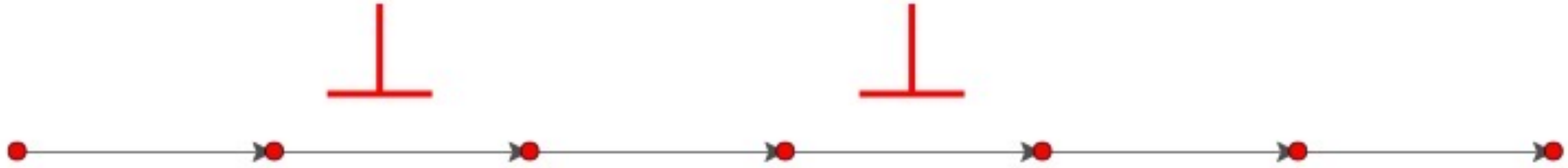
A lengthy linear series of steps
is criticallly susceptible to blockage at any step



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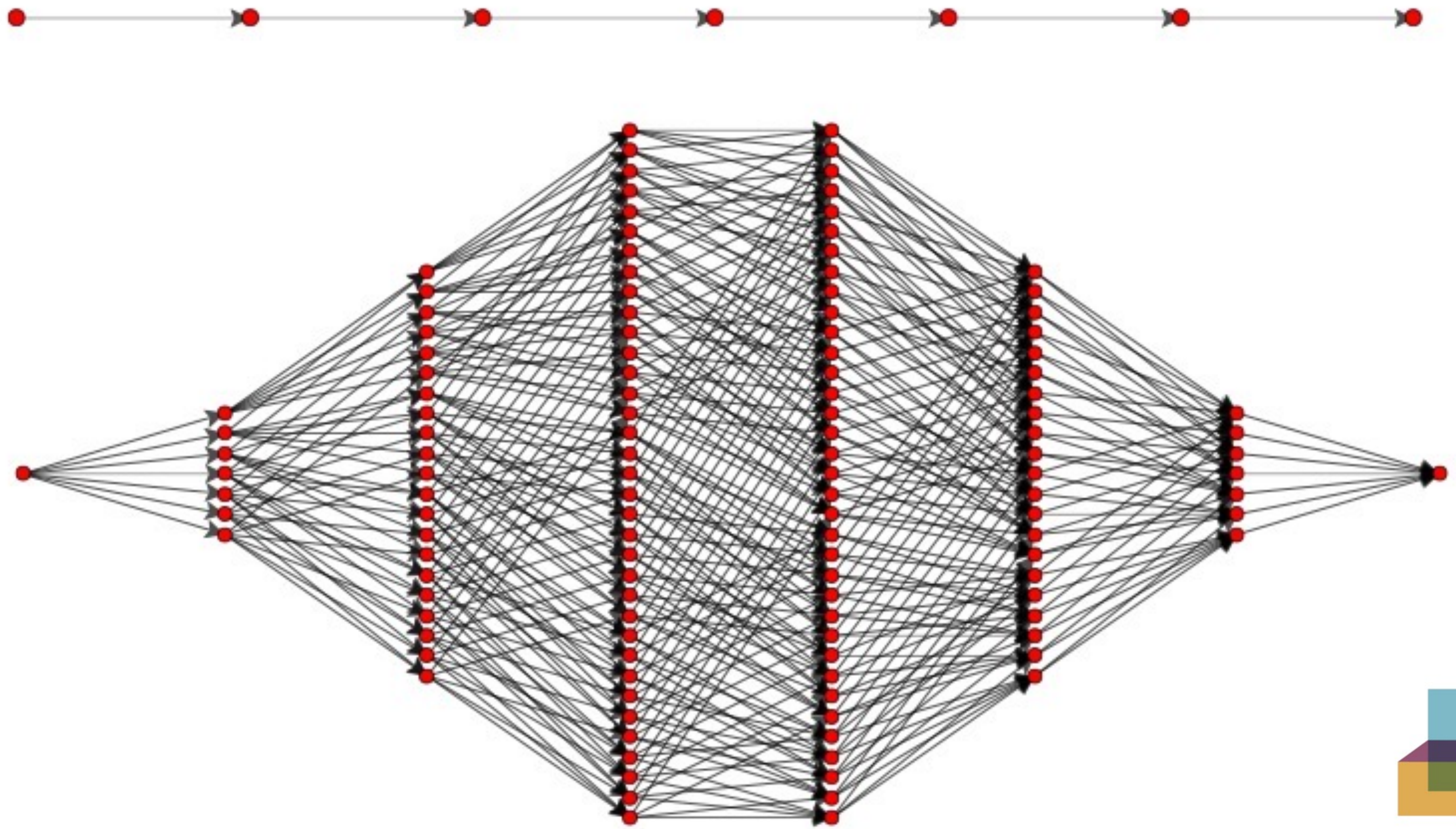
# Random assembly order leads to a combinatorial explosion of intermediates



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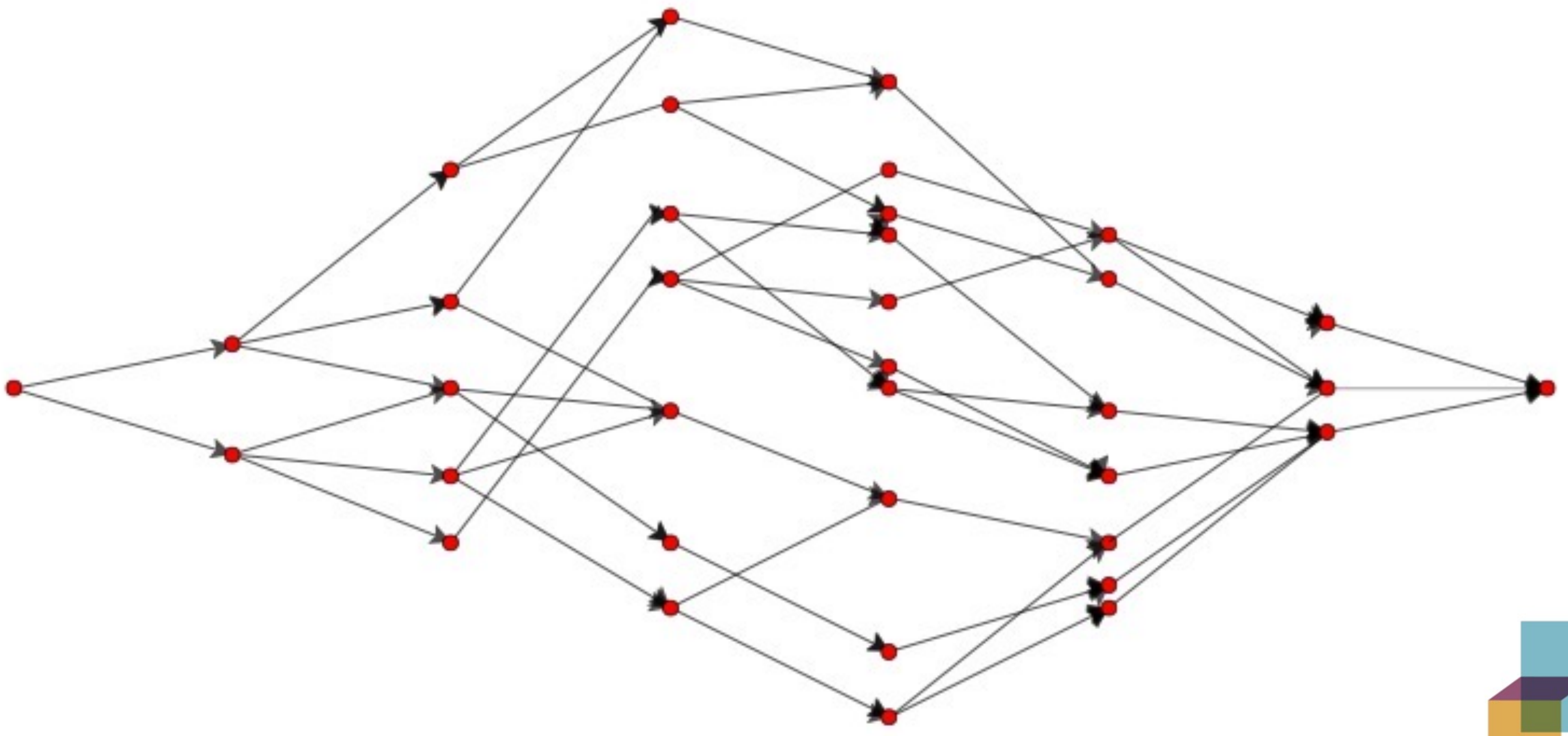
# Ribosome Assembly has evolved to produce a balance between parallel and sequential steps

- flexibility without chaos



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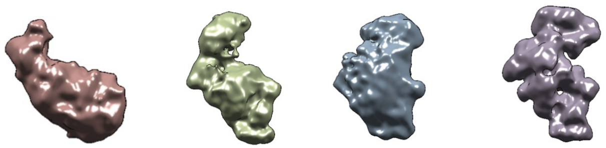
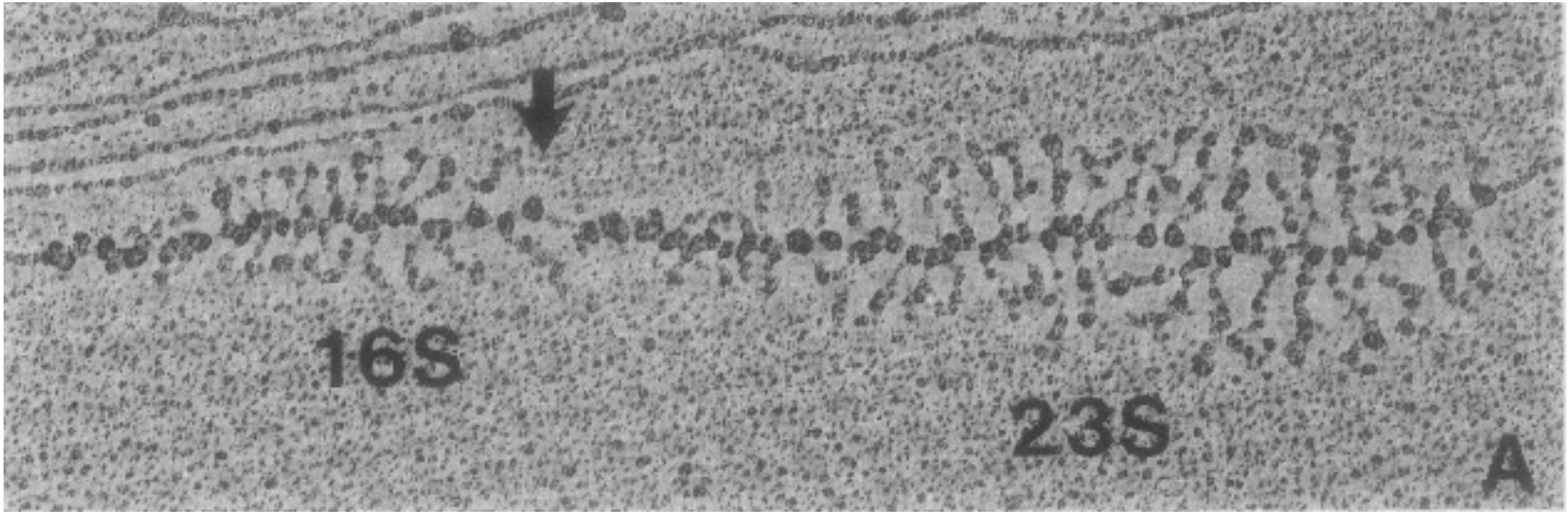
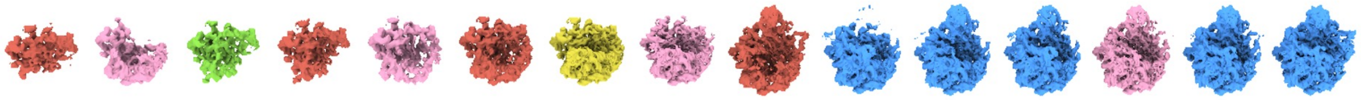
# Approaching a clear structural picture of the intermediates in assembly



30S intermediates




50S intermediates



*Next Frontier: Ultrastructure of the rRNA operon in cells*

- cryo-electron tomography with Danielle Grotjahn!

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Static Picture… …Dynamic Process

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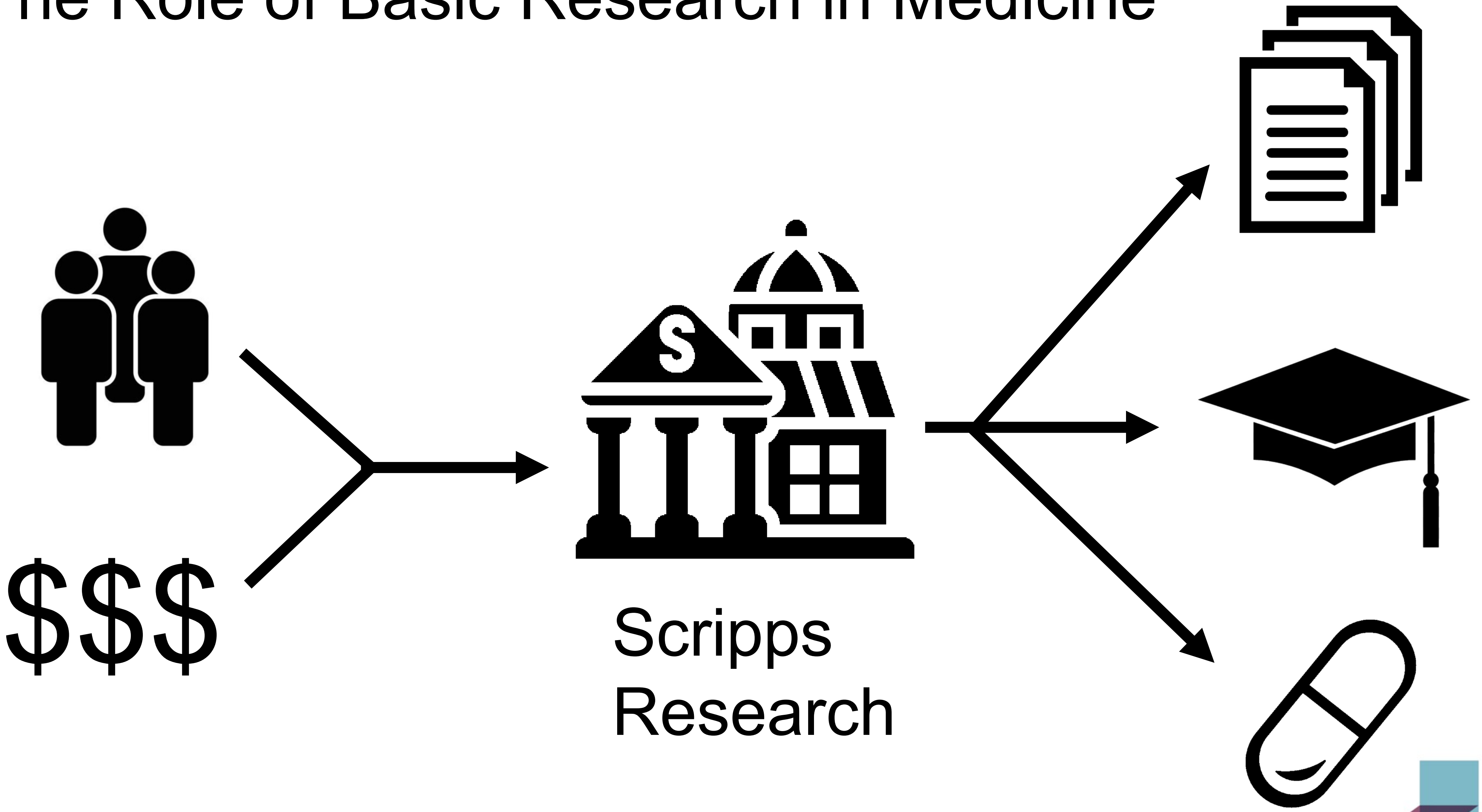
Static Picture… …Dynamic Process

# The Role of Basic Research in Medicine

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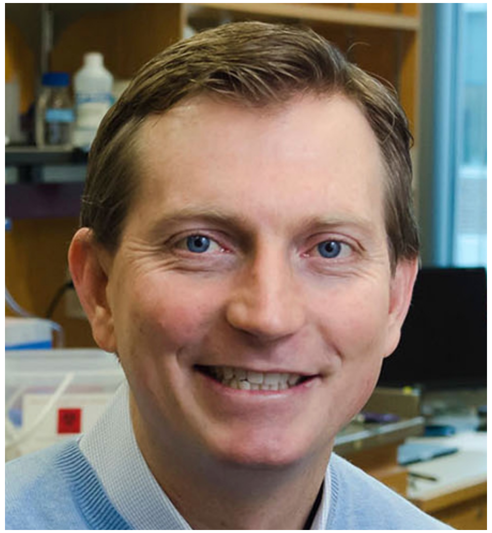
# Basic Research Fuels Academia and Biotechnology



Joey Davis

Philip Zamore

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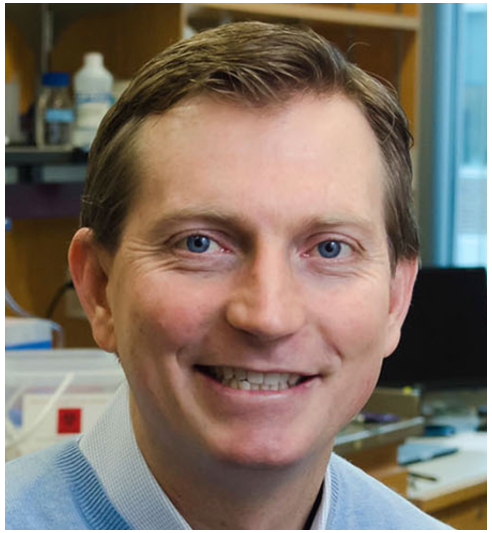
# Basic Research Fuels Academia and Biotechnology



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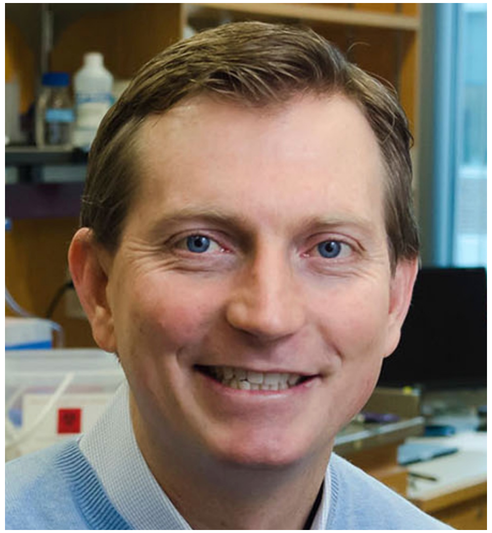

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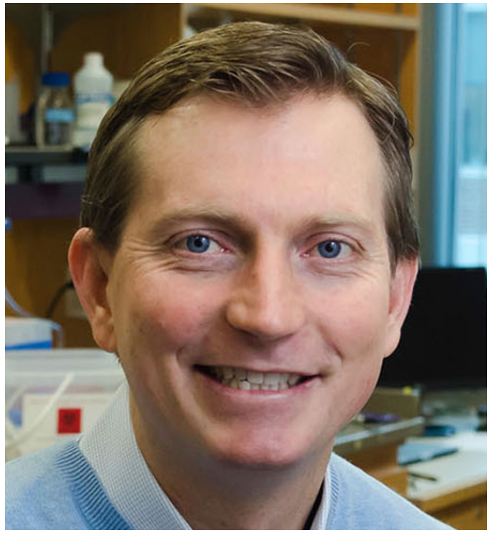

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Philip Zamore

Biochemistry Chair
UMass Medical Center

Founder of *Alnylam Inc.*
RNA Therapeutics
$27B Market Cap

Patrick Zarrinkar

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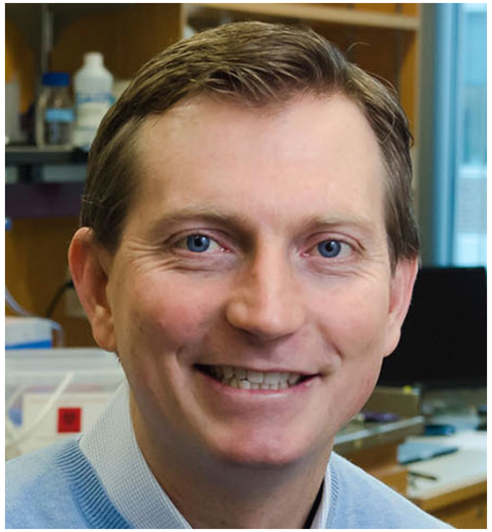
# Basic Research Fuels Academia and Biotechnology



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Patrick Zarrinkar

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Recludix Pharma
San Diego, CA

Daniel Treiber

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Recludix Pharma
San Diego, CA

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# Ribosome Assembly and Disease:

- Some diseases are due to defects in ribosome assembly –
  - Diamond Blackfan Anemia (DBA)
  - Treacher Collins Syndrome (TCS)
  - Shwachman Diamond Syndrome (SDS)

- Some anticancer drugs work by inhibiting ribosome assembly

- Some antibiotics work by inhibiting ribosome assembly

- All these drugs were discovered by random screening….
  only later was the mechanism uncovered

- Our work provides the opportunity for discovery of specific inhibitors
  based on understanding the mechanism of ribosome assembly

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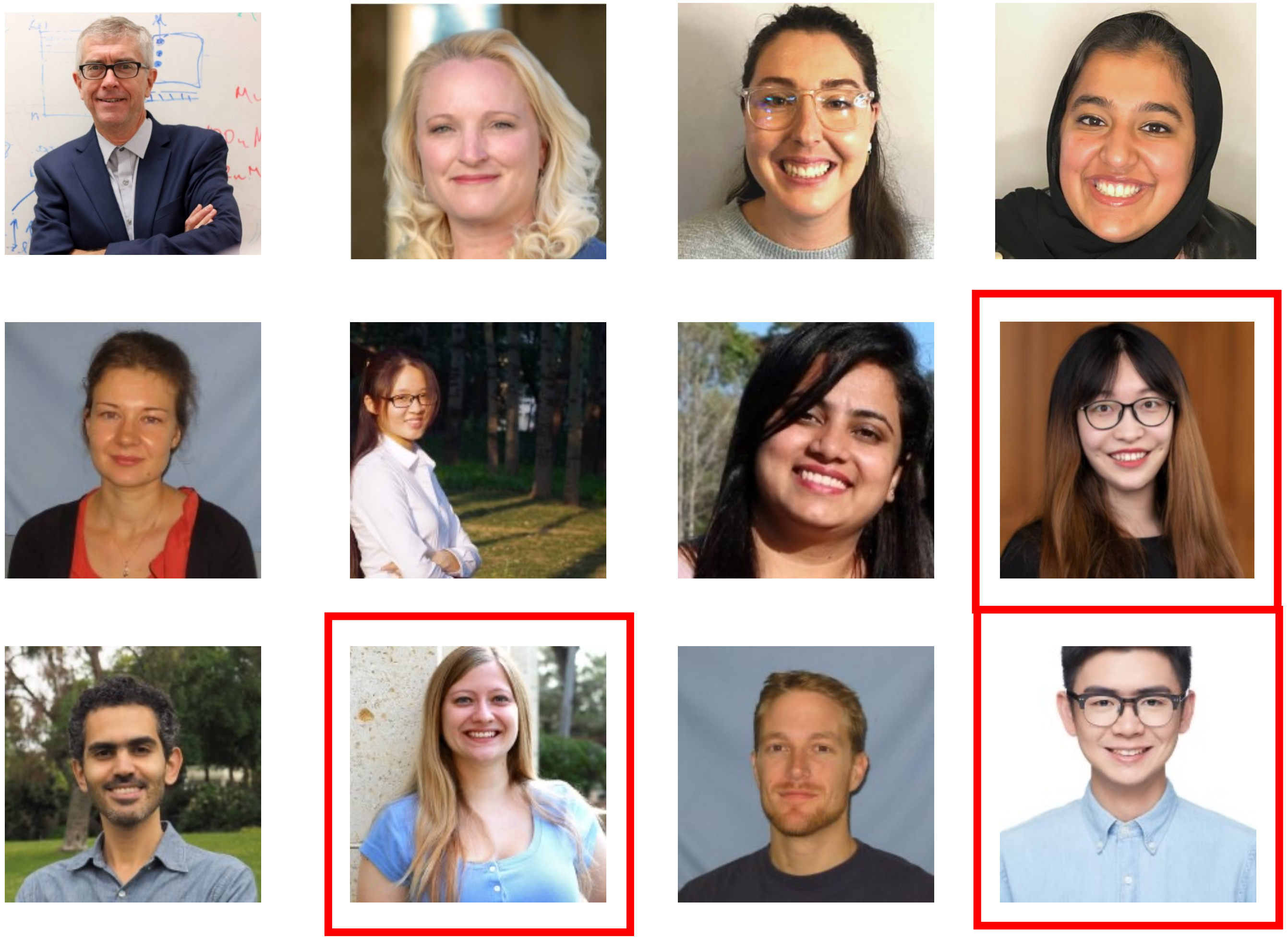

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