Quantitative Microbiology

PHYS 176/276
Instructor: Terry Hwa
Winter 2023

course website:
https://matisse.ucsd.edu/courses/w23-quant-microb/

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What is quantitative biology?

➔ quantitative biology ≠ biology + numbers/equations
≠ application of quant tools to bio

➔ use numbers to gain predictive understanding of living systems

Why quantitative biology?

- because biology *is* quantitative
- needed to formulate and test falsifiable predictions
- demanded by synthetic biology

Role of theory

- formulate expectation and predictions (via quantitative model)
- guide the design of new experiments and technology
- power: the generality of (falsifiable) ideas, not necessarily math [e.g., Cupernicus, Darwin, Einstein]
- "cost" : the simplifying assumptions, not necessarily forced by math, but required in order to reveal principles

➔ This course: quantitative (molecular) microbiology

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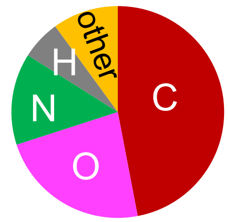
❖ Life of a bacterium:

matter + energy ➔ biomass

TABLE 1. Typical elemental composition of biological specimen

Element	Mass fraction in the following cells	
	Tissue[a]	Bacteria[b]
C	0.50	0.47
N	0.16	0.14
H	0.07	0.06
O	0.25	0.23
P + S + others	0.02	0.10[c]

[Heldal et al, 1985]



- molar composition: $CH_{1.5}O_{0.35}N_{0.24}$ (+S, P, Mg, Fe, …)
- algae (photosynthesis):
  $CO_2 + H_2O + N_2$ + photons ➔ biomass + O_2
- *E. coli* (minimal medium):
  glucose + NH_3 ➔ biomass + CO_2

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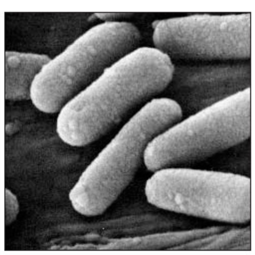
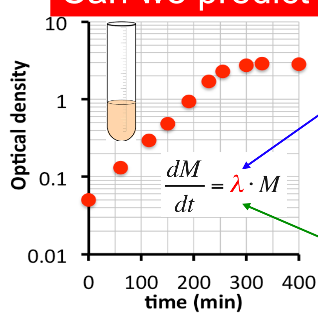
growth of E. coli

Can we predict GR & yield?





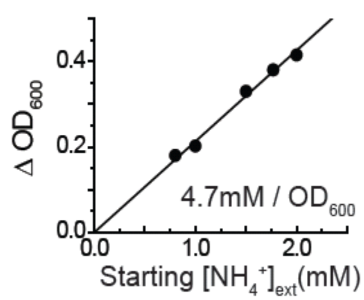
environmental factors:
nutrient types & conc
temperature, pH,
osmolarity, drugs, …

genetic factors:
enzymes & regulation

Learning from the growth curve

[Monod, Ann Rev Microb. 1949]

- OD_{600} = biomass content
  [1 OD·ml = 0.5mg CDW ~ 10^9 cells]
- saturation OD ➔ yield
- (lag: transition from pre-shift phase)



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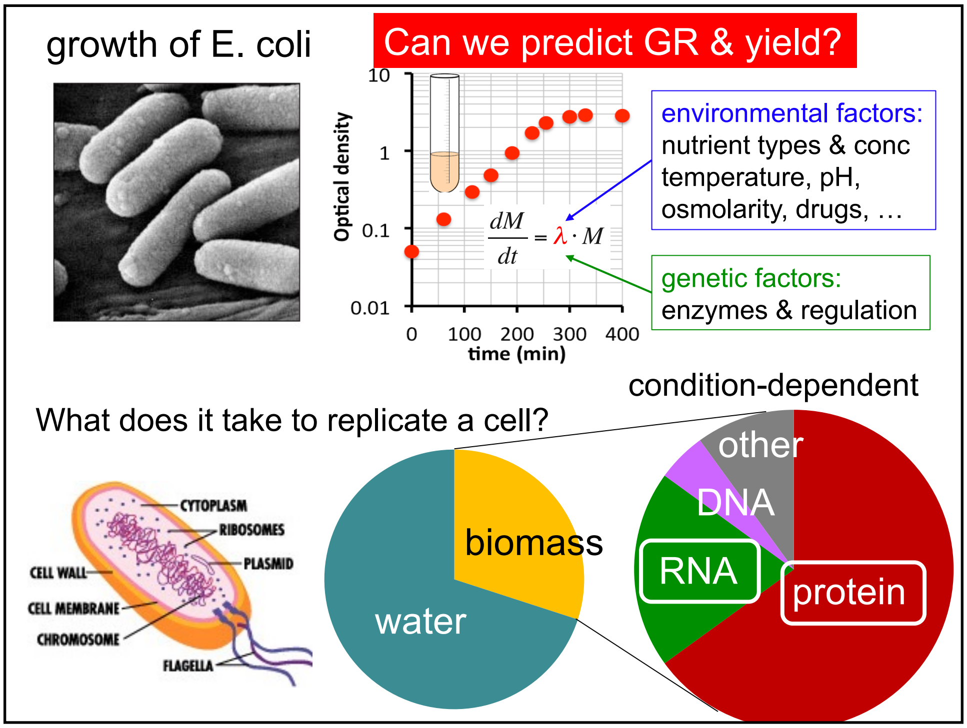
growth of E. coli
Can we predict GR & yield?
Optical density
10
1
0.1
0.01
0
100
200
300
400
time (min)
dM/dt = λ · M
environmental factors:
nutrient types & conc
temperature, pH,
osmolarity, drugs, …
genetic factors:
enzymes & regulation
What does it take to replicate a cell?
CYTOPLASM
RIBOSOMES
PLASMID
CELL WALL
CELL MEMBRANE
CHROMOSOME
FLAGELLA
biomass
water
condition-dependent
other
DNA
RNA
protein

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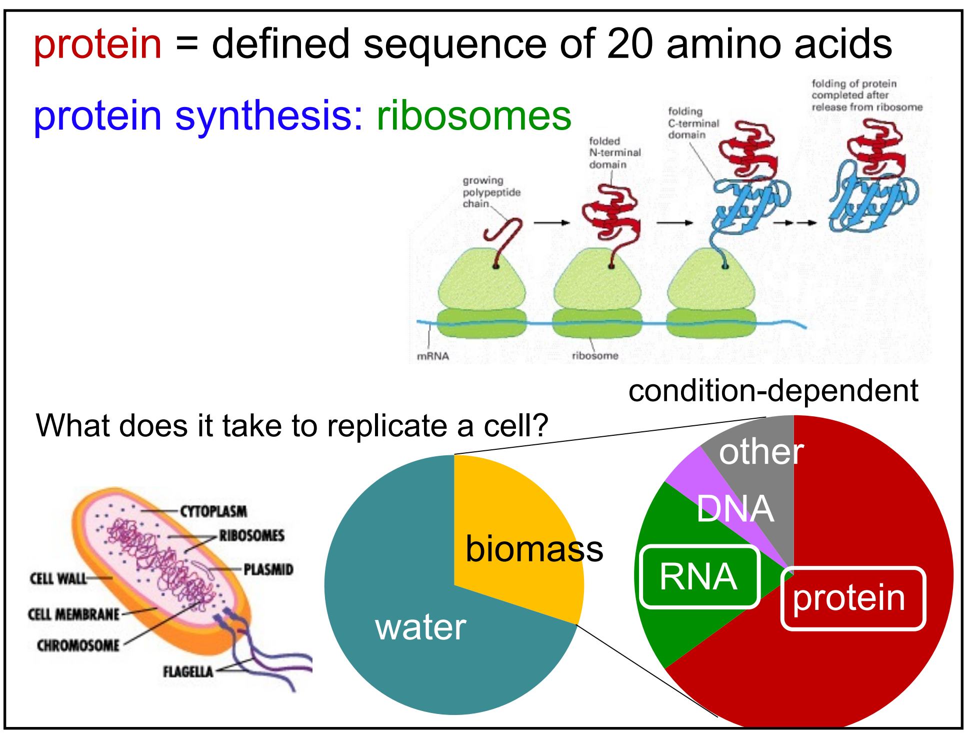
protein = defined sequence of 20 amino acids
protein synthesis: ribosomes
growing polypeptide chain
folded N-terminal domain
folding C-terminal domain
folding of protein completed after release from ribosome
mRNA
ribosome
What does it take to replicate a cell?
CYTOPLASM
RIBOSOMES
PLASMID
CELL WALL
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biomass
water
condition-dependent
other
DNA
RNA
protein

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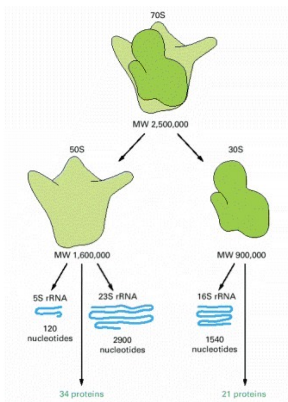
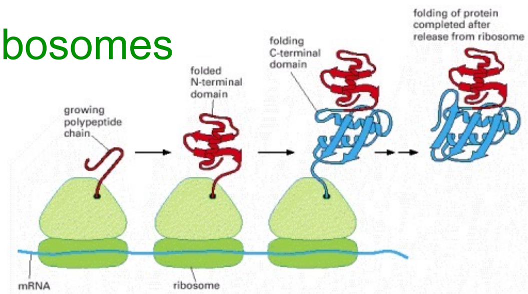
protein = defined sequence of 20 amino acids

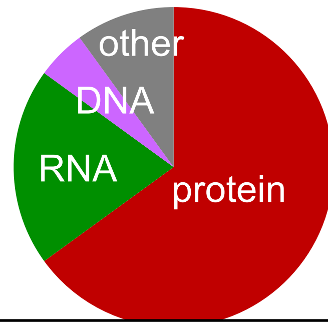
protein synthesis: ribosomes





>85% of all RNA
up to 1/3 of all proteins

condition-dependent



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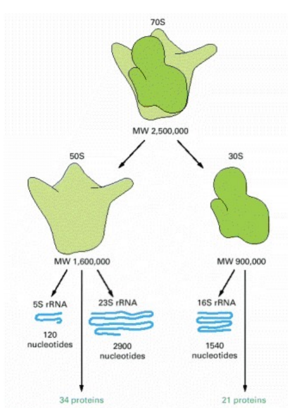
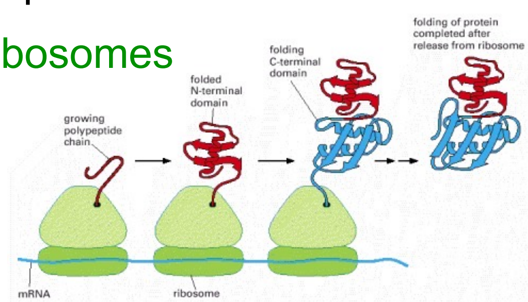
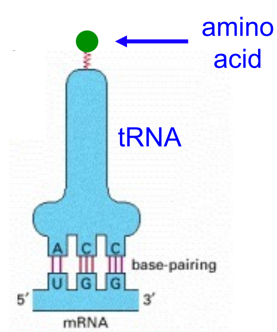
protein = defined sequence of 20 amino acids

protein synthesis: ribosomes

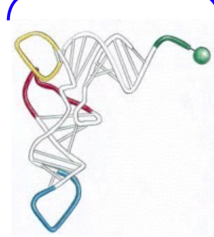




>85% of all RNA
up to 1/3 of all proteins



charged tRNA



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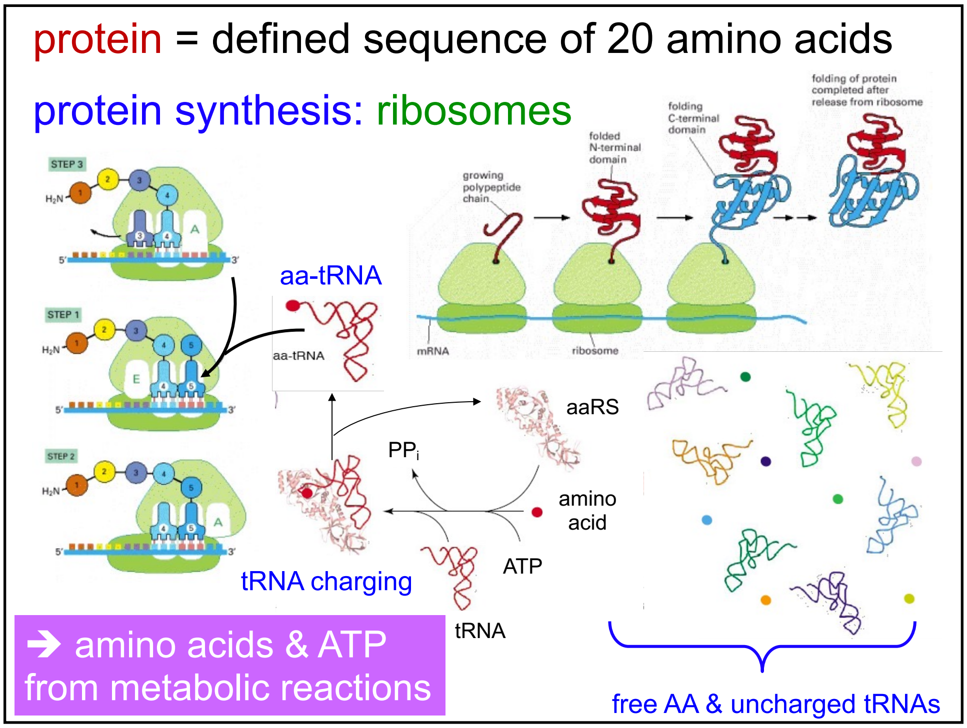
protein = defined sequence of 20 amino acids
protein synthesis: ribosomes
STEP 3
STEP 1
STEP 2
aa-tRNA
aa-tRNA
growing polypeptide chain
folded N-terminal domain
folding C-terminal domain
folding of protein completed after release from ribosome
mRNA
ribosome
aaRS
PPi
amino acid
ATP
tRNA charging
tRNA
→ amino acids & ATP from metabolic reactions
free AA & uncharged tRNAs

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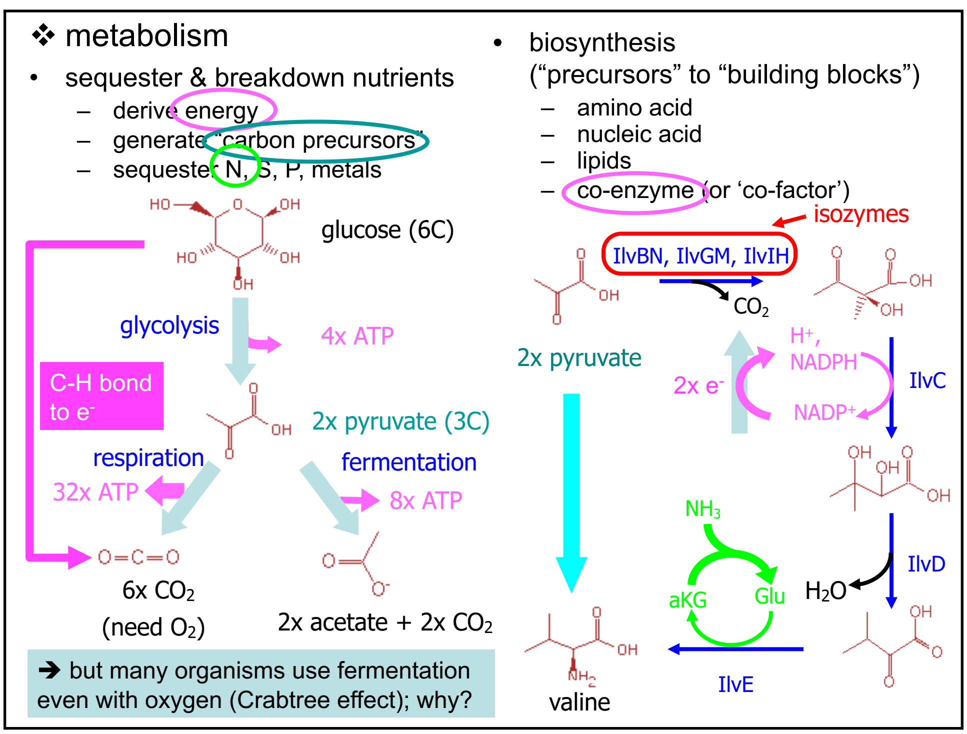
❖ metabolism
• sequester & breakdown nutrients
– derive energy
– generate "carbon precursors"
– sequester N, S, P, metals
glucose (6C)
glycolysis
4x ATP
C-H bond to e-
2x pyruvate (3C)
respiration
32x ATP
fermentation
8x ATP
6x CO2
(need O2)
2x acetate + 2x CO2
→ but many organisms use fermentation even with oxygen (Crabtree effect); why?
• biosynthesis ("precursors" to "building blocks")
– amino acid
– nucleic acid
– lipids
– co-enzyme (or 'co-factor')
isozymes
IlvBN, IlvGM, IlvIH
CO2
2x pyruvate
H+, NADPH
2x e-
NADP+
IlvC
NH3
IlvD
aKG
Glu
H2O
IlvE
valine

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❖ metabolism

- sequester & breakdown nutrients
  - derive energy
  - generate carbon ... s
  - sequester N, ... metals
- biosynthesis of building blocks
  - amino acid
  - nucleic acid
  - lipids
  - co-enzymes
- degradation/recycling (e.g., mRNA)
- typical biochemical reaction:

  $S + C \bullet b \rightleftarrows S \bullet b + C$

  S: substrate

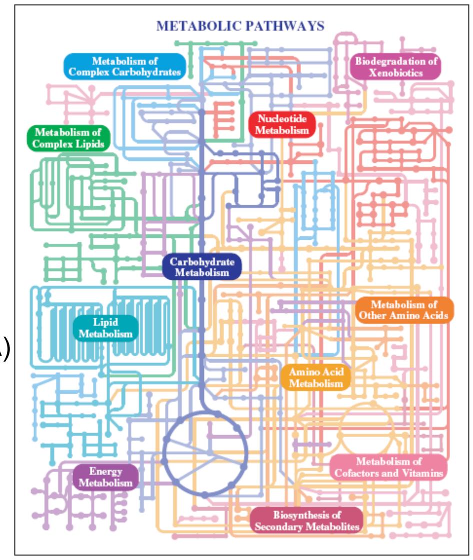
  b: component (e.g., CH_3, NH_2, e^-)

  C: co-enzyme

  (needed for difficult reactions)

catabolism

anabolism



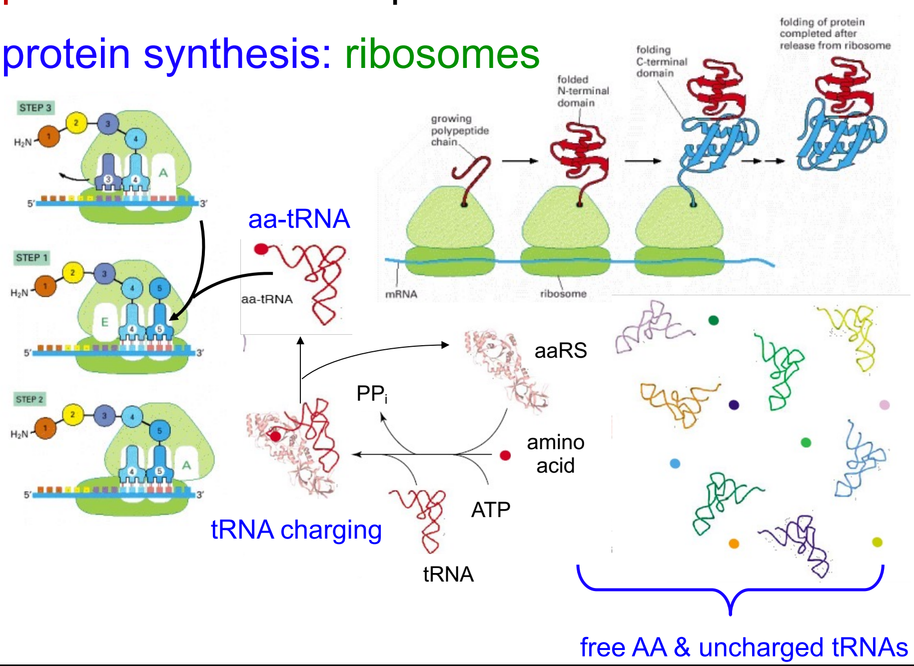
➔ most reactions catalyzed by enzymes (proteins)

➔ flux of the products and “by-products” need to be balanced

metabolic control via <u>coordinated regulation</u> of enzyme abundance/activity

protein = defined sequence of 20 amino acids

protein synthesis: ribosomes



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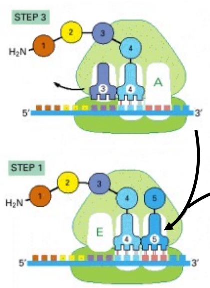
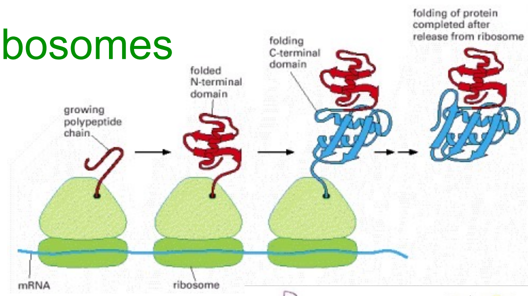


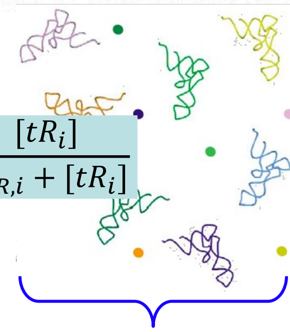


aa-tRNA

tRNA charging flux

~100 variables
~500 parameters

$$J_i = k_i[RS_i]\frac{[a_i]}{K_{a,i} + [a_i]} \cdot \frac{[tR_i]}{K_{tR,i} + [tR_i]}$$



free AA & uncharged tRNAs

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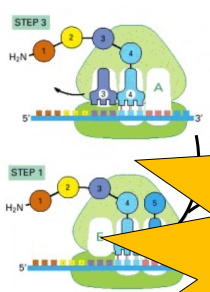
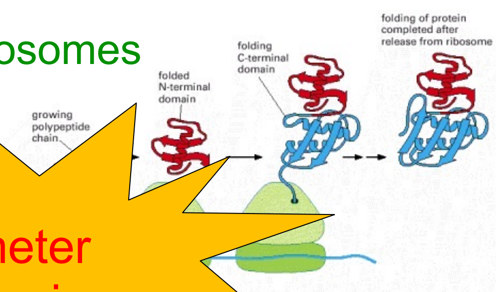
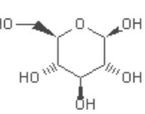
protein = defined sequence of 20 amino acids

protein synthesis: ribosomes





parameter explosion



glucose (6C)

~100 variables
~500 parameters

~500 enzymes
~500 metabolites
~5000 parameters

~10000 parameters

regulation: when and how much proteins to make

dependence on temp, pH, osmolarity, …

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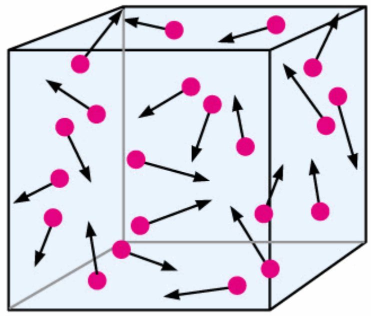
How to deal with exploding no. of parameters?



Newtonian Mechanics → Thermodynamics

dimension reduction?

$$\frac{d^2\vec{r}_i}{dt^2} = \vec{f}_{ij}(\vec{r}_i - \vec{r}_j)$$

$$PV = nRT$$

Need moles of parameters:

$\vec{r}_i(t=0) = \ ...$

$\vec{v}_i(t=0) = \ ...$

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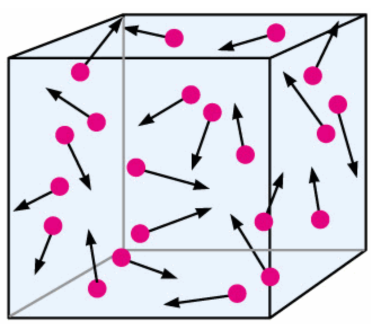
How to deal with exploding no. of parameters?



Newtonian Mechanics → Thermodynamics

dimension reduction?

Statistical Mechanics

$$\rho(\vec{q}_i, \vec{p}_i; t) \xrightarrow{t\to\infty} \rho_{eq}(\mathcal{H}(\vec{q}_i, \vec{p}_i))$$

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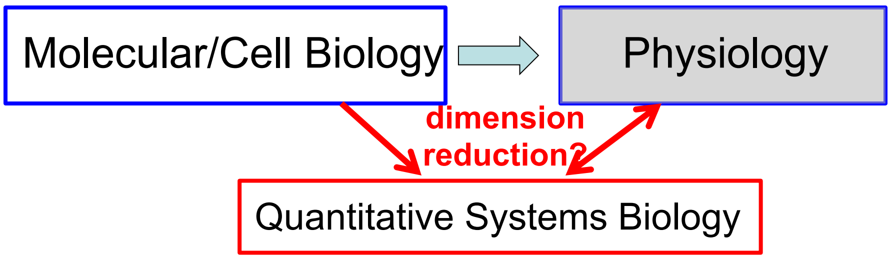
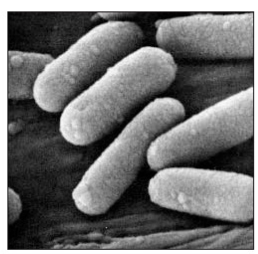
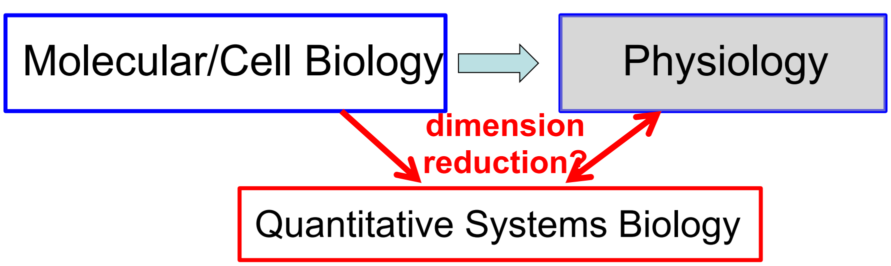
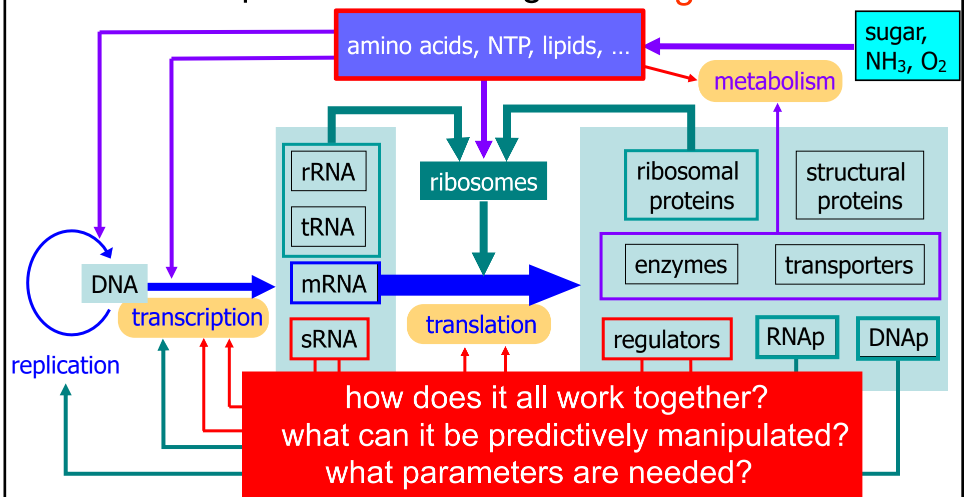






This course: explore cellular strategies of dimension reduction using bacterial mechanisms

Molecular aspect: Central Dogma + regulation



- tsx initiation control by transcription factors (TF)
- tsl initiation control by sRNA and RNA-binding proteins
- tsx termination control by sRNA and anti-terminators
- control of mRNA and protein degradation
- control of enzyme activity by metabolites

} coupled to environmental signals; coord growth program

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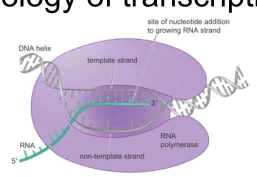
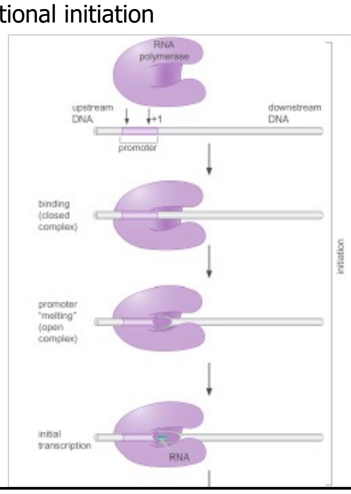
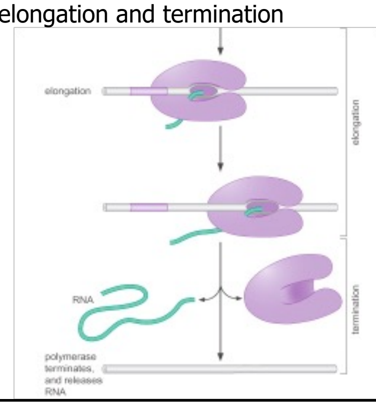
molecular biology of transcription (RNA synthesis)



transcriptional initiation



elongation and termination



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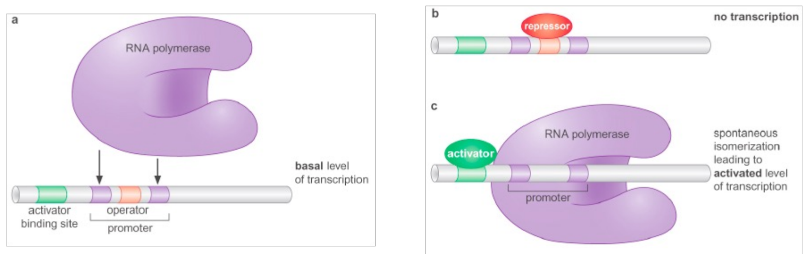
transcriptional initiation control

- modulation of RNAp-promoter affinity
  via activators and repressors



➔ net result: rate of transcriptional initiation dependent on cellular conc of activators and repressors controlled by metabolites and signaling molecules

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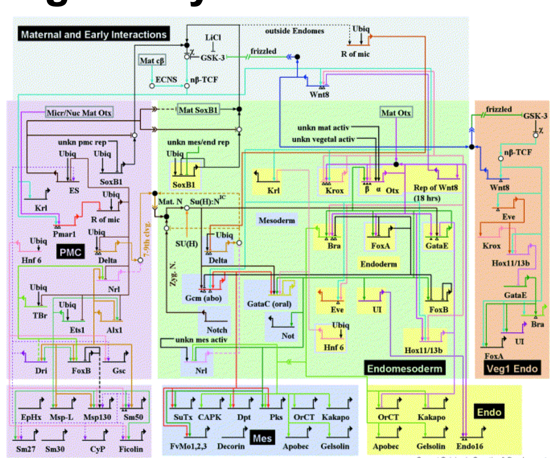
Gene regulatory networks



- ➢ A grand challenge of Systems Biology
  - – map out the complete wiring diagram of the cell
  - – predictive computational model of the cell

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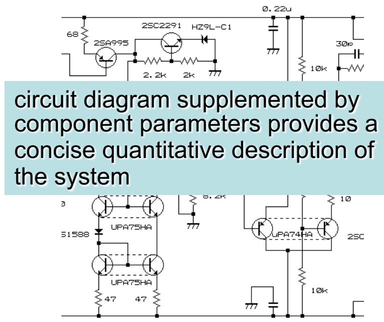
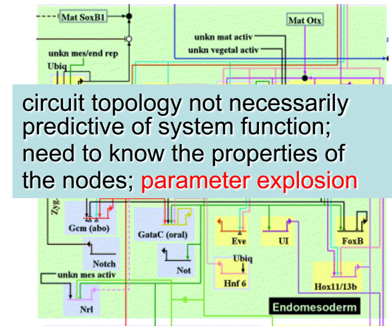
Circuit diagram as system-level descriptor ?



circuit diagram supplemented by component parameters provides a concise quantitative description of the system



circuit topology not necessarily predictive of system function; need to know the properties of the nodes; parameter explosion

	electronic circuits	genetic circuits
components	simple & well-characterized; many (~10^9); fast (10^{-9} sec)	heterogeneous, most rates unknown; few (~1000); slow (>10 min)
connectivity	physical interconnect between well-insulated components (1~2 inputs per node)	multiply-connected (1~10 inputs per node); regulation at all stages
network complexity	iterated cascades from complex network wiring	combinatorial signal integration from complex molecular control

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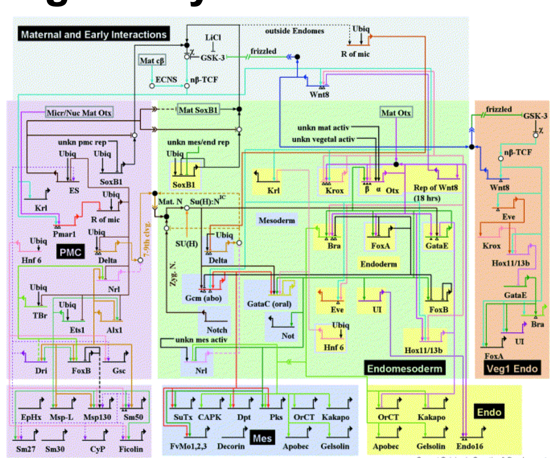
Gene regulatory networks



- A grand challenge of Systems Biology
  - map out the complete wiring diagram of the cell
  - predictive computational model of the cell

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scope of this course

- focus on simple systems (bacterial gene regulation)
- role of theory, modeling, and computation
- coarse-grained description at multiple scales (telescoped description)
- **quantitative connections between molecular mechanisms and physiological (functional or behavioral) characteristics**
- **power of functional and mechanistic constraints**

Course content

– molecular interactions: ligand-protein, protein-DNA, and protein-protein

– transcriptional control: activation, repression, and combinatorial

– modeling genetic circuits: bi-stability, oscillation, and stochasticity

– post-transcriptional control and functional enhancement

– from molecular interaction to cell physiology

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