Topic 1B: Review of Molecular Biology
What is life?

Energy

Self-replicating chemical system
Cells compute:

Can we decipher the biological hardware and software?

(Andrianantoandro, Basu, Karig, Weiss (2006))
Some Overarching Organizational Rules:

• Biological parts self-assemble

• Biological parts are actively transported

• Biological parts interact specifically

These interactions lead to complex spatial and temporal patterns that produce the variety of life

complex behaviour arising within complex interacting systems = the language of physics
The nature and size of things in biology
Two Generic Cell Types:

**Bacteria**
- Gram-positive
- Gram-negative
- Purple bacteria
- Cyanobacteria
- Flavobacteria
- Thermotogales

**Archaea**
- Methanobacteria
- Methanococcus
- Pyrococcus
- Thermoproteus
- T. celare
- Halophiles

**Eukaryotes**
- Entamoebae
- Slime molds
- Fungi
- Plants
- Ciliates
- Flagellates
- Trichomonads
- Diplomonads
- Mircosporea

**Us**
- Animals

**Higher Organisms**
- DNA organized in multiple chromosomes inside a nucleus. Mitotic division.

**Prokaryote**
- DNA organized in a single chromosome. No nucleus. No mitosis.
Prokaryotic Cells:

- **Thick Walled**: (Staphylococcus, Streptococcus, anthrax)
- **Thin Walled**: (the plague, salmonella, meningitis, E. coli, cholera)

**Penicillin**

**Bad pathogens**

**Thick Walled**

**Thin Walled**
Eukaryotic Cells:

The mitochondria make energy – very important.
E. Coli – our biological ruler

Figure 2.1b Physical Biology of the Cell, 2nd ed. (© Garland Science 2013)

Figure 2.1c Physical Biology of the Cell, 2nd ed. (© Garland Science 2013)
Huge variety in cells:
Setting some length scales

bases

DNA

viral capsid

bacteriophage

E. coli

0.1 nm

1 nm

10 nm

0.1 μm

E. coli

ZOOMING OUT

1 μm

10 μm

100 μm

1000 μm

epithelial cell

epithelium

tissue

Figure 2.15 (part 1 of 2) Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Figure 2.15 (part 2 of 2) Physical Biology of the Cell, 2ed. (© Garland Science 2013)
Table 1.1: Rules of thumb for biological estimates.

<table>
<thead>
<tr>
<th>Quantity of interest</th>
<th>Symbol</th>
<th>Rule of thumb</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. coli</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell volume</td>
<td>$V_{E.\text{coli}}$</td>
<td>$\approx 1 \mu m^3$</td>
</tr>
<tr>
<td>Cell mass</td>
<td>$m_{E.\text{coli}}$</td>
<td>$\approx 1 \text{ pg}$</td>
</tr>
<tr>
<td>Cell cycle time</td>
<td>$t_{E.\text{coli}}$</td>
<td>$\approx 3000 \text{ s}$</td>
</tr>
<tr>
<td>Cell surface area</td>
<td>$A_{E.\text{coli}}$</td>
<td>$\approx 6 \mu m^2$</td>
</tr>
<tr>
<td>Macromolecule concentration</td>
<td>$c_{\text{macromol}}^{E.\text{coli}}$</td>
<td>$\approx 300 \text{ mg/mL}$</td>
</tr>
<tr>
<td>in cytoplasm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genome length</td>
<td>$N_{E.\text{coli}}$</td>
<td>$\approx 5 \times 10^6 \text{ bp}$</td>
</tr>
<tr>
<td>Swimming speed</td>
<td>$v_{E.\text{coli}}$</td>
<td>$\approx 20 \mu m/s$</td>
</tr>
<tr>
<td><strong>Yeast</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume of cell</td>
<td>$V_{\text{yeast}}$</td>
<td>$\approx 60 \mu m^3$</td>
</tr>
<tr>
<td>Mass of cell</td>
<td>$m_{\text{yeast}}$</td>
<td>$\approx 60 \text{ pg}$</td>
</tr>
<tr>
<td>Diameter of cell</td>
<td>$d_{\text{yeast}}$</td>
<td>$\approx 5 \mu m$</td>
</tr>
<tr>
<td>Cell cycle time</td>
<td>$t_{\text{yeast}}$</td>
<td>$\approx 200 \text{ min}$</td>
</tr>
<tr>
<td>Genome length</td>
<td>$N_{\text{yeast}}$</td>
<td>$\approx 10^7 \text{ bp}$</td>
</tr>
<tr>
<td><strong>Organelles</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter of nucleus</td>
<td>$d_{\text{nucleus}}$</td>
<td>$\approx 5 \mu m$</td>
</tr>
<tr>
<td>Length of mitochondrion</td>
<td>$l_{\text{mito}}$</td>
<td>$\approx 2 \mu m$</td>
</tr>
<tr>
<td>Diameter of transport vesicles</td>
<td>$d_{\text{vesicle}}$</td>
<td>$\approx 50 \text{ nm}$</td>
</tr>
<tr>
<td><strong>Water</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume of molecule</td>
<td>$V_{H_2O}$</td>
<td>$\approx 10^{-2} \text{ nm}^3$</td>
</tr>
<tr>
<td>Density of water</td>
<td>$\rho$</td>
<td>$1 \text{ g/cm}^3$</td>
</tr>
<tr>
<td>Viscosity of water</td>
<td>$\eta$</td>
<td>$\approx 1 \text{ centipoise}$ (10^{-2} \text{ g/(cm s)})</td>
</tr>
<tr>
<td>Hydrophobic embedding energy</td>
<td>$E_{\text{hydr}}$</td>
<td>$2500 \text{ cal/(mol nm}^2$</td>
</tr>
</tbody>
</table>

Table 1.1 (part 1 of 2) Physical Biology of the Cell, 2ed. (© Garland Science 2013)
Table 1.1: Rules of thumb for biological estimates.

<table>
<thead>
<tr>
<th>Quantity of interest</th>
<th>Symbol</th>
<th>Rule of thumb</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length per base pair</td>
<td>$l_{bp}$</td>
<td>$\approx 1/3 , \text{nm}$</td>
</tr>
<tr>
<td>Volume per base pair</td>
<td>$V_{bp}$</td>
<td>$\approx 1 , \text{nm}^3$</td>
</tr>
<tr>
<td>Charge density</td>
<td>$\lambda_{DNA}$</td>
<td>$2 \frac{e}{0.34 , \text{nm}}$</td>
</tr>
<tr>
<td>Persistence length</td>
<td>$\xi_p$</td>
<td>$50 , \text{nm}$</td>
</tr>
<tr>
<td>Amino acids and proteins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radius of “average” protein</td>
<td>$r_{protein}$</td>
<td>$\approx 2 , \text{nm}$</td>
</tr>
<tr>
<td>Volume of “average” protein</td>
<td>$V_{protein}$</td>
<td>$\approx 25 , \text{nm}^3$</td>
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<tr>
<td>Mass of “average” amino acid</td>
<td>$M_{aa}$</td>
<td>$\approx 100 , \text{Da}$</td>
</tr>
<tr>
<td>Mass of “average” protein</td>
<td>$M_{protein}$</td>
<td>$\approx 30,000 , \text{Da}$</td>
</tr>
<tr>
<td>Protein concentration in cytoplasm</td>
<td>$c_{protein}$</td>
<td>$\approx 150 , \text{mg/mL}$</td>
</tr>
<tr>
<td>Characteristic force of protein motor</td>
<td>$F_{motor}$</td>
<td>$\approx 5 , \text{pN}$</td>
</tr>
<tr>
<td>Characteristic speed of protein motor</td>
<td>$v_{motor}$</td>
<td>$\approx 200 , \text{nm/s}$</td>
</tr>
<tr>
<td>Diffusion constant of “average” protein in cytoplasm</td>
<td>$D_{protein}$</td>
<td>$\approx 10 , \mu m^2/s$</td>
</tr>
<tr>
<td>Lipid bilayers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thickness of lipid bilayer</td>
<td>$d$</td>
<td>$\approx 5 , \text{nm}$</td>
</tr>
<tr>
<td>Area per molecule</td>
<td>$A_{lipid}$</td>
<td>$\approx \frac{1}{2} , \text{nm}^2$</td>
</tr>
<tr>
<td>Mass of lipid molecule</td>
<td>$m_{lipid}$</td>
<td>$\approx 800 , \text{Da}$</td>
</tr>
</tbody>
</table>

Table 1.1 (part 2 of 2) Physical Biology of the Cell, 2nd ed. (© Garland Science 2013)
The stuff inside: small molecules

- **water** – we’re 70% H2O
- ions: H+, Na+, Ca2+, K+, etc. used to drive transport, pumps
- **sugars** – glucose, ribose, sucrose – substrates for many metabolic reactions
- **nucleotides** – form DNA & RNA
  - ATP (adenosine triphosphate – the big energy source of cells)
- **amino acids** – form proteins
- **fatty acids** – chain like molecules that are the basis of lipids that make the membrane
Lipids & Membranes: The packaging

- Lipids are molecules which form membrane bi-layers
- consist of two fatty acids joined by a ‘head’ molecule of glycerol
- head group likes water, tail does not like water
- Lipids spontaneously self-assemble into membranes
Lipids & Membranes: a physics view

Figure 1.7 Physical Biology of the Cell, 2ed. (© Garland Science 2013)
DNA:

Four different nucleotides: A, C, T, G

A = T
G = C

Watson-Crick Base Pairing

PYRIMIDINES
- Thymine

PURINES
- Adenine
- Cytosine
- Guanine
Figure 1.3b Physical Biology of the Cell, 2nd ed. (© Garland Science 2013)
The Structure of DNA in Cells:

• How do you pack 1 m of DNA into a nucleus that is 2 μm in size?

2nm DNA gets wrapped around histone complexes forming what are called nucleosomes (DNA –vely charged & histones are +vely charged)

These then wrap up and pack further and condense
RNA:

- Alphabet = G, C, A, U (instead of T)

- Secondary structure

- Tertiary structure

- Carry the genetic information around as mRNA

- Can carry out chemical functions
Proteins: Amino Acids

- Proteins are polymers built from 20 amino acids

![Side Chain Diagram]

![Amino Acid Molecules Diagram]
Proteins: Structure

Secondary Structure
Helices & Strands

Tertiary Structure
Densely packed hydrophobic core

Amino acid 1 + Amino acid 2 → Peptide bond

NH₂ – C – C – O
R₁

Amino terminus

R₂

Peptide bonds

HOOC

Carboxy terminus
Information Storage, Replication & Processing:

• What does the cell use all these molecules/structure for?
  1) Storing Chemical Information
  2) Passing On/Replicating this information
  3) Processing and calculating with this information

We'll look at each of these steps now
Cellular “states”:

- Cellular state is determined by which genes=proteins are “ON”
- Different cell types = different genetic programs that are being run
Information Storage:

• The information for running the cellular program is stored in the sequence of DNA.

• DNA is like the hard drive of a computer.

• It stores information, contains programs that get executed at specific times.

• Each protein has a specific sequence in the DNA called a ‘gene’, and this DNA is called ‘coding’ DNA, as it codes for messages that make proteins.

• The majority of DNA sequence does not code for ‘genes’ and is called non-coding DNA.

• It is the non-coding DNA which contains the ‘programs’ which determine what genes will get made at a given time or place.

• Different cells are running different programs and thus making different sets of genes.
The Sizes of the Hard-drives

Bacteria (usually) have single circular chromosome – have many small plasmids

Eukaryotes have multiple linear chromosomes

- **haploid** = single copy of every chromosome
- **diploid** = two copies of every chromosome
- **polyploid** = multiple copies of every chromosome

<table>
<thead>
<tr>
<th>organism</th>
<th>#</th>
<th>T</th>
<th>size</th>
<th>genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>virus</td>
<td>1</td>
<td>H</td>
<td>5kb</td>
<td>10</td>
</tr>
<tr>
<td>E. coli</td>
<td>1</td>
<td>H</td>
<td>5Mb</td>
<td>4,377</td>
</tr>
<tr>
<td>S. Cervisiae</td>
<td>16</td>
<td>H</td>
<td>12Mb</td>
<td>5,570</td>
</tr>
<tr>
<td>C. elegans</td>
<td>16</td>
<td>D</td>
<td>100Mb</td>
<td>19,000</td>
</tr>
<tr>
<td>A. thaliana</td>
<td>5</td>
<td>D</td>
<td>115Mb</td>
<td>25,498</td>
</tr>
<tr>
<td>D. melanogaster</td>
<td>4</td>
<td>D</td>
<td>122Mb</td>
<td>14,000</td>
</tr>
<tr>
<td>H. sapiens</td>
<td>23</td>
<td>D</td>
<td>3Gb</td>
<td>25,000</td>
</tr>
</tbody>
</table>
Figure 3.12 Physical Biology of the Cell, 2ed. (© Garland Science 2013)
Information Replication: Cell Division:

Asexual and normal cell division

MITOSIS

- DNA Replication
- Cell Division

Sex cells = gametes = haploids

Meiosis

1st cell division of meiosis

- Prophase 1
- Metaphase 1
- Anaphase 1
- Telophase 1

2nd cell division of meiosis

- Prophase 2
- Metaphase 2
- Anaphase 2
- Telophase 2

Parent cell

paternal homologue
maternal homologue

2 daughter cells

4 daughter cells
DNA replication:

Replication Times:

bacteria ~ 40 mins (1 origin)
1000 nucleotides/s

humans ~ few hours (many origins)
100 nucleotides/s
Information Processing: Central Dogma:

Replication

DNA → DNA

Transcription

DNA → RNA

Translation

RNA → Protein
DNA to Proteins? Genetic Code

- 64 codons, with highly non-uniform mapping
Gene Transcription:

- Bacteria = ON
- Eukaryotes = OFF, requires lots of other help

Pushes polymerase off of DNA
Messages are delivered differently:

Prokaryotes (Simple):

- Genes in bacteria are organized in operons – one contiguous message RNA is generated

Eukaryotes (Complex):

- Genes contain coding (exons) and non-coding (introns) sequence
- Introns are spliced out and exons are assembled to make final mRNA
- final mRNA is exported out of nucleus and made into protein
Translation:

- Ribosome is a big machine that translates mRNA into protein.
- In bacteria, the ribosome binds to a specific site on mRNA.
- In eukaryotes, the ribosome binds to the 5' cap.
- Translation ends when the 'STOP' codon is reached.
Figure 3.13 Physical Biology of the Cell, 2ed. (© Garland Science 2013)
How cells get energy

- Oxidation of food $\rightarrow$ CO2 and H2O and energy
- **Metabolism** = processes of converting/synthesizing molecules in cells
  - **Catabolism** = breaking down of molecules
  - **Anabolism** = synthesizing of molecules
- Two major energy reserves: **sugars**, **fatty acids**
Most chemical reactions in cells are mediated by proteins called **enzymes**.

Enzymes act as catalysts, by reducing the activation barrier between two states.
Energy Storage in Cells

- Some chemical reactions in cells are energetically unfavourable (e.g., DNA & protein synthesis).
- Couple energetically bad pathway with favourable pathway using enzyme.
- Favourable pathway is driven by taking energy from energy carriers.
- Most pervasive energy carrier is ATP.

\[
\text{ATP} \rightarrow \text{ADP} + P + \text{H}_2\text{O} + (13 \text{ kcal/mol} = 25 \text{ kT})
\]

- DNA synthesis burns 2 ATP.

Energy from burning of food \(\rightarrow\) store in ATP \(\rightarrow\) burn ATP to drive reactions.
Converting Food to Useful Energy:

**Glycolysis**
- anaerobic
- converts glucose $\rightarrow$ 2 ATP

**Citric Acid Cycle = Krebs Cycle:**
- aerobic
- occurs in the mitochondria
- generates about 30 ATP from a single glucose!!!
- convert about 50% of possible energy (c.f. car ~ 20%)

---

**Diagram:**
- LIVER: glucose $\rightarrow$ pyruvate $\rightarrow$ lactate
- MUSCLE: glucose $\downarrow$ pyruvate $\downarrow$ lactate
- BLOOD connects the liver and muscle.
Numbers of things in cell biology
Why knowing numbers in biology matters?

- Given the vast range of scales and the large variety of parts, it's important to have a sense of the numbers of things.
- **Why?**
  1. Being able to estimate numbers of parts etc. builds intuition.
  2. Allows one to assess what aspects of a given biological process will be important quantitatively.

- In complex systems, the things one ignores are as important as the things that are considered.
- Models are always incomplete.

Roadmap to Quantitative Biology:

- Experiment → Quantitative Data → Intuition & model building
  \[ \frac{d^2 \theta}{dt^2} = 0 \]
  \[ F = ma \]
Figure 2.4 Physical Biology of the Cell, 2ed. (© Garland Science 2013)
Some estimates of parts:

- **Volume**: \( V_{cell} \approx 1 \, \mu m^3 = 1 \, FL \)
- **Area**: \( A_{cell} \approx 6 \, \mu m^2 \)

**Cells are crowded:**

**Mass**: 
\[
Mass = \text{density} \times Volume
\]
\[
= \text{density} \times V_{cell}
\]
\[
= 1g/mL \times 1\,FL = 1 \, pg
\]

**Dry mass**: Experiment \( \Rightarrow \) \( M_{dry} \approx 0.8 \, pg = 30\% \, Mass \)

**Protein mass**: avg. protein = 300 amino acids (AA)

1 AA = 100 Da \( \& \) 1 Da = \( M_{protein} = 1.6 \times 10^{-24} \, kg \)

so average protein mass,

\[
M_{protein} = (300) \times (100 \, Da) \times (1.6 \times 10^{-24} \, g) \approx 5 \times 10^{-20} \, g
\]

**Number of proteins in E. coli**:

50% of dry mass is protein from experiment

\[
N_{protein} = \frac{\text{protein mass}}{\text{mass per protein}} = \frac{0.8 \, pg}{5 \times 10^{-20} \, g} \approx 3 \times 10^6
\]
Some estimates of parts

Number of Ribosomes:

Expt: 20% of protein mass is contributed by ribosomes

\[ M_{\text{ribosome}} = 2.5 \text{ MDa} \]

but \( \frac{1}{3} \) Ribosome is protein & \( \frac{2}{3} \) is rRNA

\[ N_{\text{ribosome}} = \frac{(0.2)(0.15 \text{ pg})}{830,000 \text{ Da}} = 20,000 \]

How much volume do ribosomes occupy?

\[ V_{\text{ribosome}} = \frac{4}{3} \pi (10 \text{ nm})^3 \]

\[ V_{\text{tot}} = (20,000) \frac{4}{3} \pi (10 \text{ nm})^3 \approx 10^8 \text{ nm}^3 \]

\( \approx 10 \% \) \( V_{\text{cell}} \)
Table 2.1: Observed macromolecular census of an *E. coli* cell. (Data from F. C. Neidhardt et al., Physiology of the Bacterial Cell, Sinauer Associates, 1990 and M. Schaechter et al., Microbe, ASM Press, 2006.)

<table>
<thead>
<tr>
<th>Substance</th>
<th>% of total dry weight</th>
<th>Number of molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Macromolecules</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>55.0</td>
<td>2.4 \times 10^6</td>
</tr>
<tr>
<td>RNA</td>
<td>20.4</td>
<td></td>
</tr>
<tr>
<td>23S RNA</td>
<td>10.6</td>
<td>19,000</td>
</tr>
<tr>
<td>16S RNA</td>
<td>5.5</td>
<td>19,000</td>
</tr>
<tr>
<td>5S RNA</td>
<td>0.4</td>
<td>19,000</td>
</tr>
<tr>
<td>Transfer RNA (4S)</td>
<td>2.9</td>
<td>200,000</td>
</tr>
<tr>
<td>Messenger RNA</td>
<td>0.8</td>
<td>1,400</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>9.1</td>
<td>22 \times 10^6</td>
</tr>
<tr>
<td>Lipopolysaccharide (outer membrane)</td>
<td>3.4</td>
<td>1.2 \times 10^6</td>
</tr>
<tr>
<td>DNA</td>
<td>3.1</td>
<td>2</td>
</tr>
<tr>
<td>Murein (cell wall)</td>
<td>2.5</td>
<td>1</td>
</tr>
<tr>
<td>Glycogen (sugar storage)</td>
<td>2.5</td>
<td>4,360</td>
</tr>
<tr>
<td><strong>Total macromolecules</strong></td>
<td><strong>96.1</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Small molecules</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metabolites, building blocks, etc.</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>Inorganic ions</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td><strong>Total small molecules</strong></td>
<td><strong>3.9</strong></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.1 Physical Biology of the Cell, 2ed. (© Garland Science 2013)
What is the concentration of 1 molecule in a E. coli?

Concentrations and #’s of molecules

concentration = \frac{\#}{\text{volume}}

units: \text{molar} = [M] = \frac{1 \text{ mol}}{1 \text{ liter}} = \frac{6.02 \times 10^{23}}{1 \text{ L}}

Cellular concentrations range from 1 nM \rightarrow 1 \mu M

What is concentration of 1 molecule in E. coli?

C_1 = \frac{1}{1 \text{ fl}} = \frac{1 \text{ molecule}}{1 \times 10^{-19} \text{ L}} \approx 2 \text{ nM}

So, a concentration of 2 \mu M \approx 1000 \text{ molecules in the cell}

1 \text{ nM} \sim 1 \text{ molecule inside the volume of an E. coli cell}
Cells are crowded places

Distance between molecules:

- Assume molecules sit on a square lattice in a square cell of volume \( V \)

\[
\text{spacing between molecules is } \frac{d}{\sqrt{3}}
\]

\[
c = \frac{N}{V} = \frac{N}{(N \cdot d^3)} = d^{-3}
\]

so \( \text{spacing} = d = c^{-\frac{1}{3}} \)

for \( c = 2 \mu M \Rightarrow d \approx 150 \text{ nm} \)

for \( c = (1 \times 10^6) \text{ nM} \Rightarrow d \approx 1 \text{ nm} \leftarrow \text{hardly any space left for proteins} \)

\# of proteins
The timing of things: timescales in biology
Why timescales matter?

Biology is not static, it’s dynamic!

It has dynamics over a range of timescales

Q: How do we treat physical systems that have processes that are operating over many different timescales

A: We choose a timescale of interest and only consider processes that are of the same scale. Faster processes will be considered to be at steady state - we’ll define this later.
Figure 3.1 Physical Biology of the Cell, 2ed. (© Garland Science 2013)
Gating of ion channels

[Diagram showing the gating of ion channels with time in seconds, ions, and closed/open ion channels]

Figure 3.2g Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Enzyme catalysis

[Diagram showing enzyme-catalyzed reaction with time in seconds, substrate, enzyme, and products]

Figure 3.2h Physical Biology of the Cell, 2ed. (© Garland Science 2013)
The lives of molecules: RNA lifetimes

(A) *E. coli*

(B) *S. cerevisiae*

Figure 3.14 Physical Biology of the Cell, 2ed. (© Garland Science 2013)
The lives of molecules: Protein lifetimes

Figure 3.15a Physical Biology of the Cell, 2ed. (© Garland Science 2013)
Timing estimates

How fast is the replication machinery?

- There are $5 \times 10^6$ bp in E.coli.
- Thus, $\text{rate}_{\text{bp}} = \frac{5 \times 10^6 \text{ bp}}{3000 \text{ s}} = 2000 \text{ bp/s}$

- In E.coli, 2 DNA polymerases replicate the DNA from 1 origin in opposite directions.
- A single DNA polymerase in E.coli has a rate of 1000 bp/s.

Aside: In higher organisms, DNA polymerase rate ~ 100bp/s and the genomes are much larger.

Q: How do the DNA ever get replicated in time?
Timing estimates:

What is the rate of protein synthesis?

Previously, \( \approx 3 \times 10^6 \) proteins in an E.coli cell

\[
\text{rate protein} = \frac{3 \times 10^6}{3000} = 1000 \text{ proteins/s}
\]

Q: what are the mechanical properties of these bio-machines (DNA polymerase, ribosomes) that allow them to operate at these rates?

Q: given the # of ribosomes in E. coli, how many proteins per second is ribosome making?
In many biological processes, time is relative.

All that matters is that a task gets completed.

So in the cell cycle there are many checkpoints.
Figure 3.16 Physical Biology of the Cell, 2ed. (© Garland Science 2013)
Building an oscillator

General Design of Biological Oscillators

- Negative feedback + time delay can produce oscillations.

The production and degradation of cyclins depends upon the abundance of cellular resources; the absolute time of the period, T, will vary depending on the environment.
Biophysical Experimental Techniques:
FLUORESCENCE MICROSCOPY

Figure 2.13a Physical Biology of the Cell, 2ed. (© Garland Science 2013)
Figure 2.13c Physical Biology of the Cell, 2ed. (© Garland Science 2013)
Figure 4.11 Physical Biology of the Cell, 2ed. (© Garland Science 2013)
Figure 4.12 Physical Biology of the Cell, 2ed. (© Garland Science 2013)
Molecular Biology Experimental Methods:
Chromatography:

- Chromatography is used to separate and purify molecules from a complex mixture.

Types:
- **Charge** = ion-exchange chrom.
- **Hydrophobicity** = hydrophobic chrom.
- **Size** = gel-filtration chrom.
- **Binding** = affinity chrom.

Affinity chromatography can be used to elute very pure protein samples:
- DNA for DNA binding proteins
- Antibodies for specific protein
- Protein binding partners
- Engineered tags (HIS, GST)
  
  = Co-immune precipitation = CoIP
Gel Electrophoresis

- Used to determine size of biomolecules
- -vely charged molecules move in applied field through gel

- For proteins, SDS(detergent) is used to denature proteins and give them net negative charge = SDS-PAGE

- 2D SDS-PAGE can be used to sort proteins by both size and native charge
DNA manipulation

- Use **restriction enzymes** to cut specific nucleotide sequences (e.g. HpaI = GTTAAC; EcoRI = GAATTC)
- Some make blunt ends, some make dangling 'sticky' ends

```
GGCCTCG
CCGGAGCTTAA
```
```
AATTCTCGAC
GAGCTG
```

- Used to chop up large DNA segments (i.e. Chromosome)
- Used to ligate (join) different DNA fragments -> genetic engineering
Northern, Southerns and Westerns

- Used to detect quantitative amounts of specific DNA (southern), RNA (northern) or proteins (Western) from a complex mixture (i.e. cellular extract).

- Labelled DNA probe 'hybridizes' with matching partner in sample

- In westerns, a labelled antibody is used for probe
DNA Cloning

- Replicate specific DNA fragment in large quantities using bacteria
- Fragment is inserted into circular **plasmid** DNA and **transfected** into bacteria
- Large collection of bacteria colonies each with different fragment = library

- Types of libraries = genomic, cDNA
- cDNA library comes only from mRNA
- Use cDNA plasmid to express lots of specific protein = **expression vector**
Dideoxy method: use special nucleotides that stop growth of DNA
--> produces DNA that stops at specific letter positions

DNA Sequencing
Polymerase Chain Reaction (PCR):

- Use 2 DNA primers to pull out and amplify specific region of DNA in a sample

_inside the PCR reaction tube..._
Our chromosomes contain regions of 'satellite' DNA that vary in length – each individual has specific length = fingerprint

- PCR amplify regions and then use gels to measure the lengths -> yields fingerprint
Imaging the action

- Reporter
  - Green fluorescent protein (GFP), lacZ, labelled antibodies

Gene X → GFP

enhancer
Imaging II

- **In-situ hybridization** measure the presence of mRNA of specific gene
- Takes a snapshot of gene pattern – use flourescently labelled RNA probe
- Use confocal microscope to image different 2D layers & build 3D image
Detecting biomolecular interactions

**FRET (Flourescence Resonance Energy Transfer)**

- **Excitation**: 458nm
- **Energy transfer**: 480-525nm
- **Emission**: 525-575nm

**NO interaction**

**A & B interact**

---

**Yeast Two Hybrid**

**BAIT**
- Transcription Activator (TA)
  - Activation Domain (AD)
  - Binding Domain (BD)
- Promoter
- Gene

**PREY**
- AD
- Y
- BD
- Promoter
- Reporter
Interactions II:

**Phage Display:**
- Phage = virus that infects bacteria. Has an external coat of protein
- Can fuse foreign proteins to coat proteins of phage
- Used to screen libraries of proteins for specific interaction => drug design