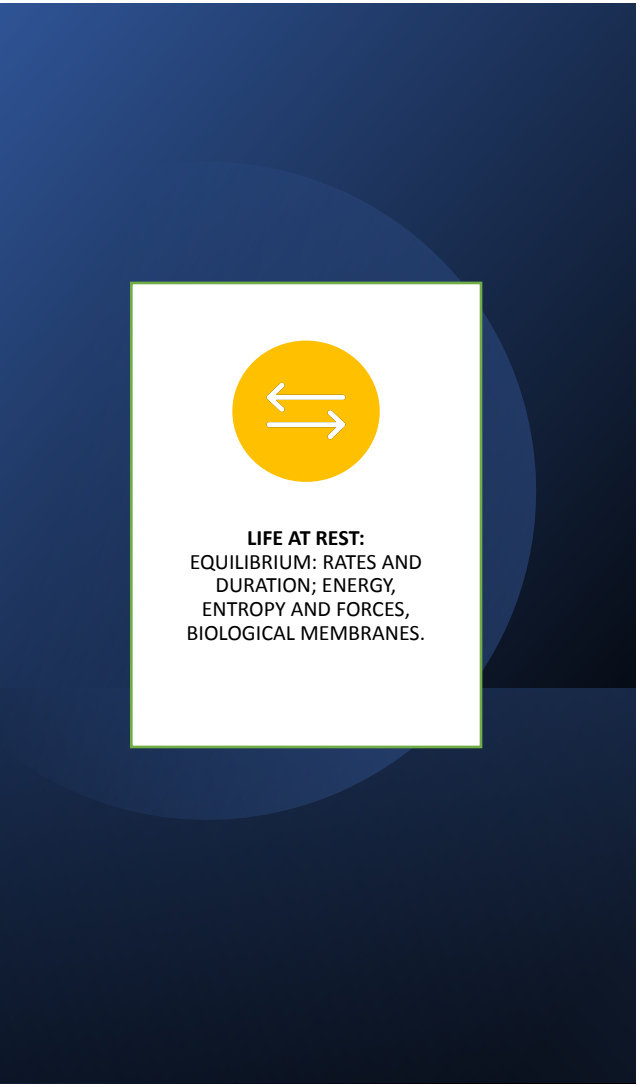
Biology for Engineers

FY-BTech

Unit 2- Life at Rest

Module-2: Life at rest



Thermodynamics and Static Properties of cells

Equilibrium: Mechanical and Chemical Equilibrium in the Living Cell; Cells as Chemical Factories; Chemical equilibrium, rate of reaction. The concept of steady state equilibrium.

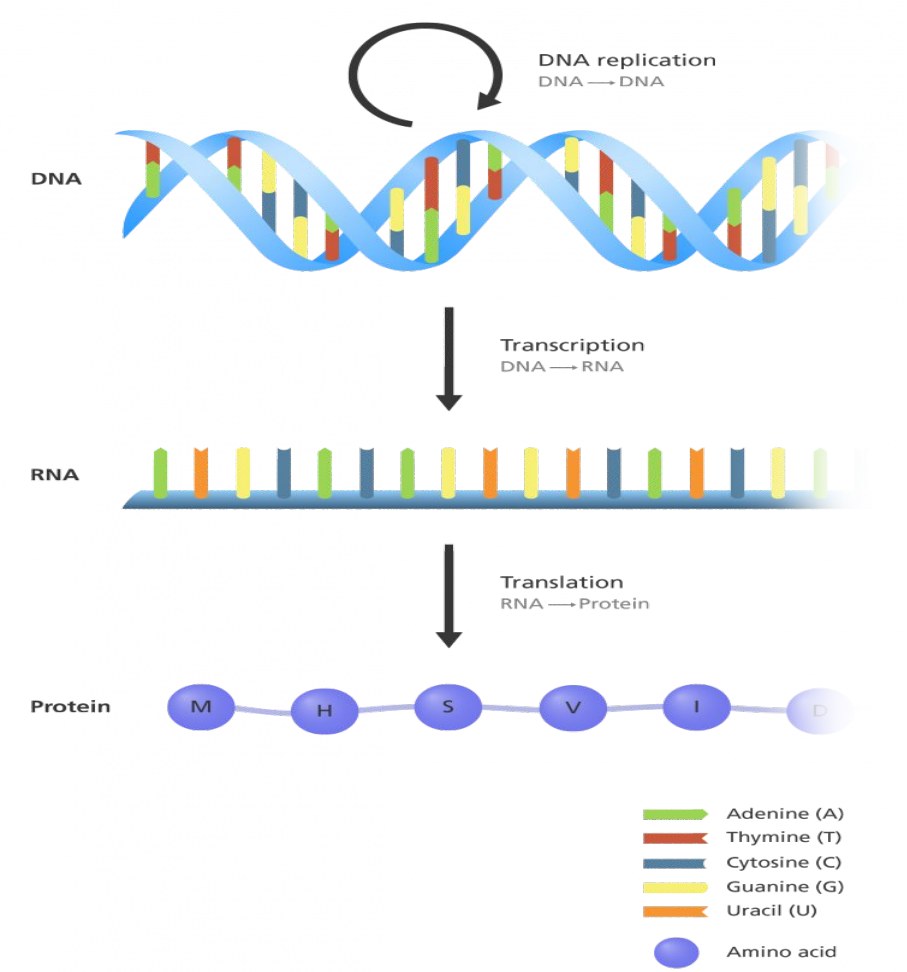
Rates and duration: Time scales of small molecules; central dogma, Life cycle of cells.

Energy, Entropy and Forces: Thermal energy, photons and photosynthesis; energy currencies and budget.

Electrostatics

Biological Membranes: membrane permeability: pumps and channels, action potential.

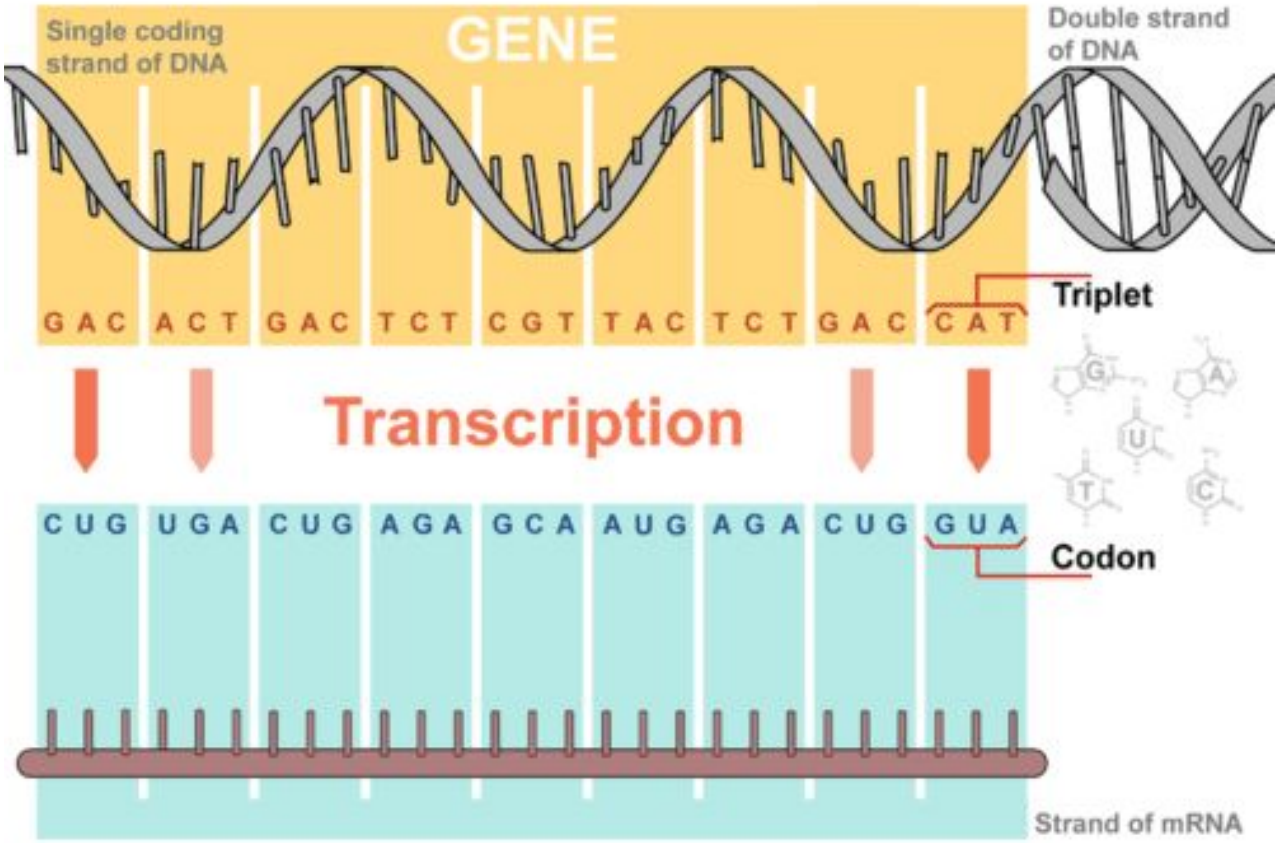
CENTRAL DOGMA



https://www.yourgenome.org/facts/what-is-the-central-dogma/

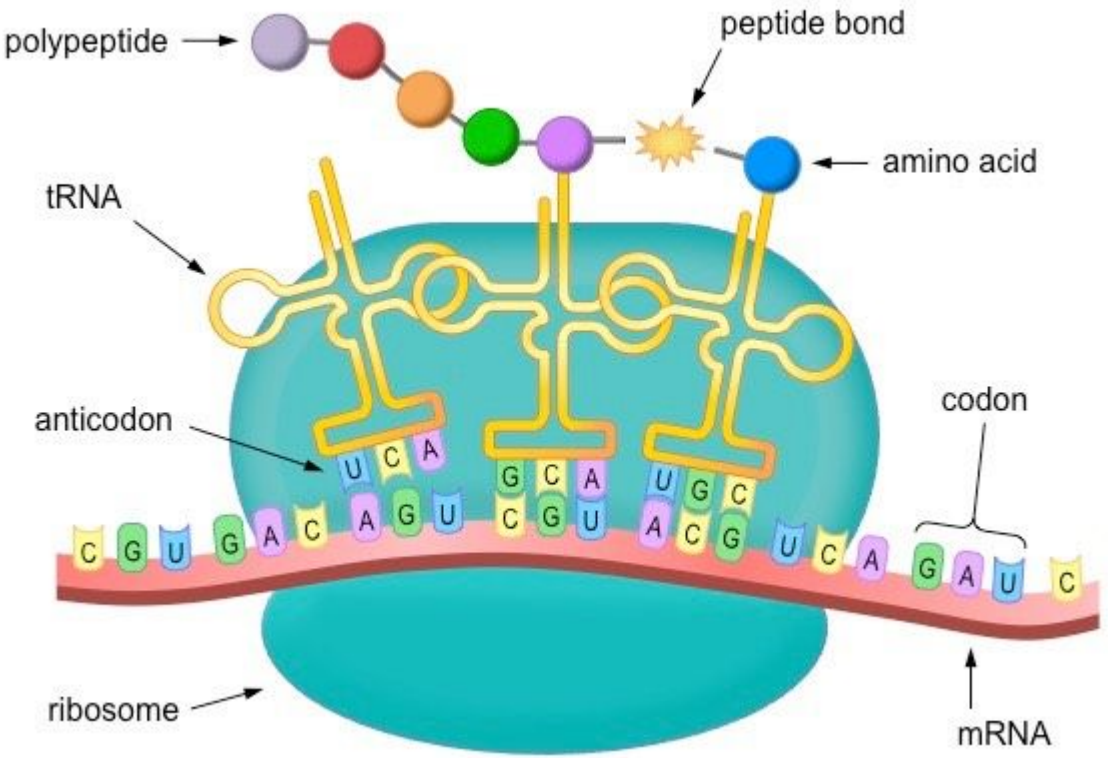
TRANSCRIPTION

The first step during protein synthesis when the DNA in a gene is copied to produce an RNA transcript called messenger RNA (mRNA).



https://courses.lumenlearning.com/wm-biology1/chapter/reading-steps-of-genetic-transcription/

TRANSLATION

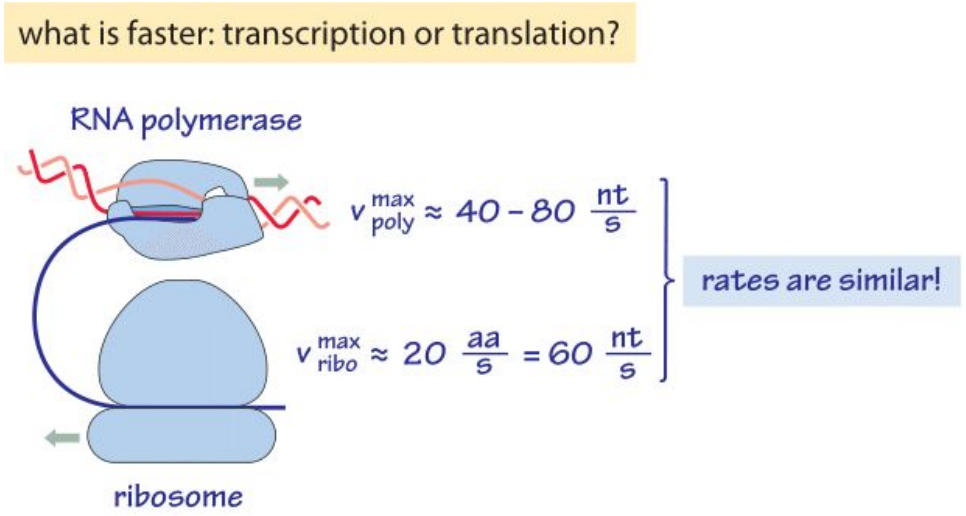


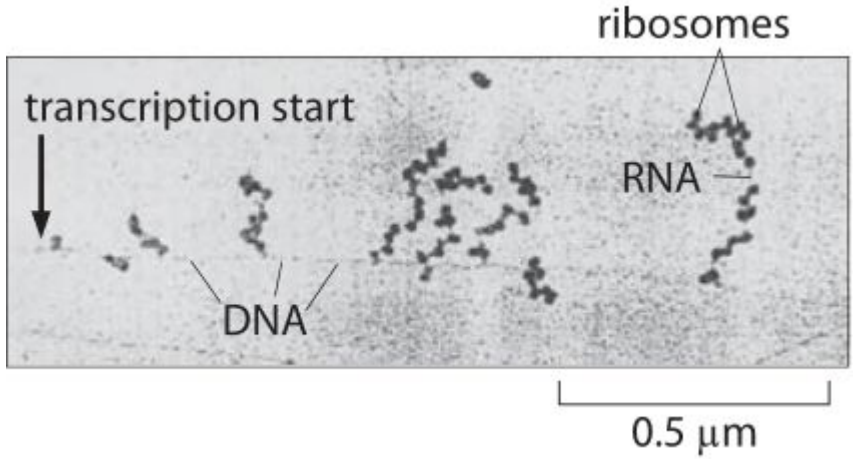
WHAT IS FASTER, TRANSCRIPTION OR TRANSLATION?

Transcription, the synthesis of mRNA from DNA, and translation, the synthesis of protein from mRNA, are the main pillars of the central dogma of molecular biology.

Transcription of RNA in E. coli of both mRNA and the stable rRNA and tRNA, is carried out by ≈1000-10,000 RNA polymerase molecules proceeding at a maximal speed of about 40-80 nt/sec.

Translation of proteins in E. coli is carried out by ≈10,000-100,000 ribosomes and proceeds at a maximal speed of about 20 aa





HOW FAST DO PROTEASOMES DEGRADE PROTEINS?

One of the ways in which the protein content of the cell is controlled is by the regulated degradation of its proteins.

The main macromolecular machine in charge of degradation is the proteasome.

What fraction of the proteome is made up of these machines?

In HeLa cells about 1% of the total bulk protein was reported to be proteasomes

In blood cells, the fraction of proteasomes out of the proteome varies between 0.01-0.3% for different cell types.

The half-life of these machines is found to be about 5 days .

The degradation rate associated with proteasome-mediated degradation is currently based on in-vitro measurements. These rates exhibit a great deal of variability with rates coming in with values from ≈0.05 through ≈0.2 to ≈5 “characteristic” peptide chains per minute.

The rate of degradation by the proteasome can vary as a function of the protein substrate.

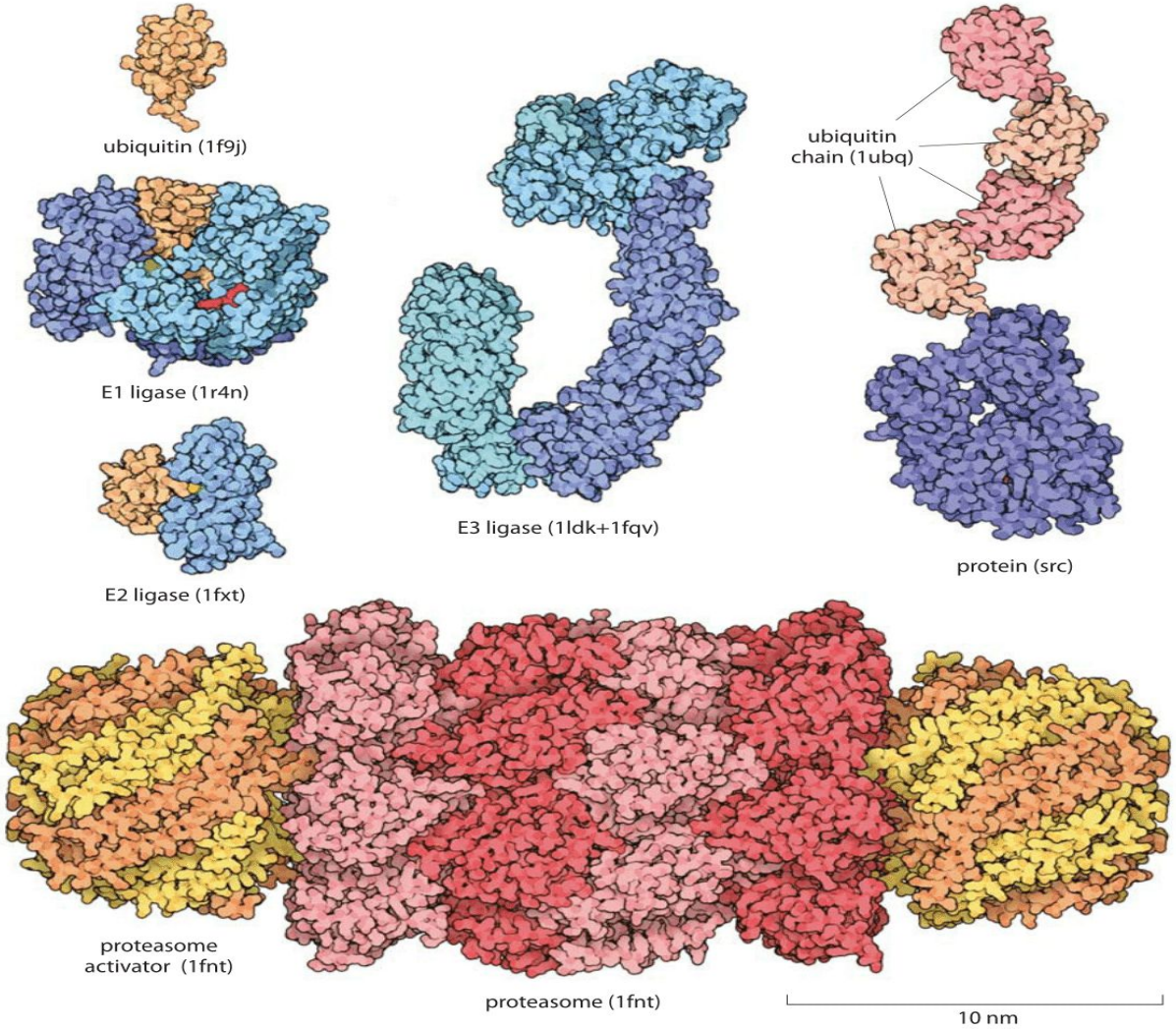


Figure 1: Proteins involved in the Ubiquitin-proteasome pathway for protein degradation. Key molecules in the degradation process range from the ubiquitin molecular tag that marks a protein for degradation to the ligases that put these molecular tags on their protein targets. Once proteins are targeted for degradation, the proteasome actively carries out this degradation. The depicted proteasome is based on the structure determined for budding yeast.

For example if there are 3 million proteins in the relevant HeLa cell of 3000 µm3 and the average length is 400 amino acids per protein then N aa is 4×10^12 aa.

Assuming ≈1% of the proteome is proteasomes, we have 0.01Naa amino acids present in those machines. The average molecular weight of a proteasome is ≈2.4×10^6 Da i.e. about 20,000 amino acids.

So there are about (0.01xNaa aa)/(20,000 aa/proteasome) ≈0.5×10-6 Naa proteasomes in the cell (i.e. on the order of a million proteasomes in the Hela cell considered above).

Taking the higher rate of proteasome degradation from above of 5 protein/min ≈ 0.1 protein/s we find that on an amino acid basis this degradation rate is equivalent to ≈40 aa/s

The rate of protein polymerization by the ribosome (≈10 aa/s) is not very far from this rate of degradation by the proteasome. The two machine complexes also share a similar molecular weight.

An overall degradation rate of (40 s-1)x(0.5×10-6 Naa aa)=20×10-6 Naa aa/s. So the turnover time, which is the total number of amino acids divided by the overall degradation rate is about Naa aa / 20×10-6 Naa aa/s ≈ 0.5×105 s, or about a day.

What is the turnover time of proteins through active degradation?

we denote the number of amino acids per cell by N_{aa}

(e.g. for HeLa cell, $N_{aa} \approx 3\times10^{6} \frac{\text{proteins}}{\mu m^3} \times 3000 \frac{\mu m^3}{\text{cell}} \times 400 \frac{\text{aa}}{\text{protein}} \approx 4\times10^{12} \frac{\text{aa}}{\text{cell}}$)

≈ 1% of proteome mass is proteasomes

$$\text{number of proteasomes} \approx \frac{0.01 \times N_{aa}}{20{,}000 \text{ aa/proteasome}} \approx 0.5\times10^{-6} N_{aa} \text{ proteasomes/cell}$$

molecular mass of proteasome ≈ 2.4 MDa ≈ 20,000 aa

proteasome deg. rate ≈ 5 proteins/min ≈ 0.1 protein/s ≈ 40 aa/s

$$\text{total deg. rate} \approx 40 \frac{\text{aa}}{\text{s x proteasome}} \times 0.5\times10^{-6} N_{aa} \frac{\text{proteasomes}}{\text{cell}} \approx 20\times10^{-6} N_{aa} \frac{\text{aa}}{\text{s x cell}}$$

$$\text{turnover time} \approx \frac{\text{number of aa per cell}}{\text{total deg rate}} \approx \frac{N_{aa} \text{ aa}}{N_{aa} \times 20\times10^{-6} \text{ aa/s}} \approx 0.5\times10^{5} \text{s} \approx \text{1 day}$$

i.e. it would take all the proteasomes working at full speed about one day to degrade all of the proteome.

HOW FAST DO RNAS AND PROTEINS DEGRADE?

Degradation is another key process in the lives of the macromolecules of the cell and is itself tightly controlled. Indeed, in the simplest model of mRNA production, the dynamics of the average level of mRNA is given by

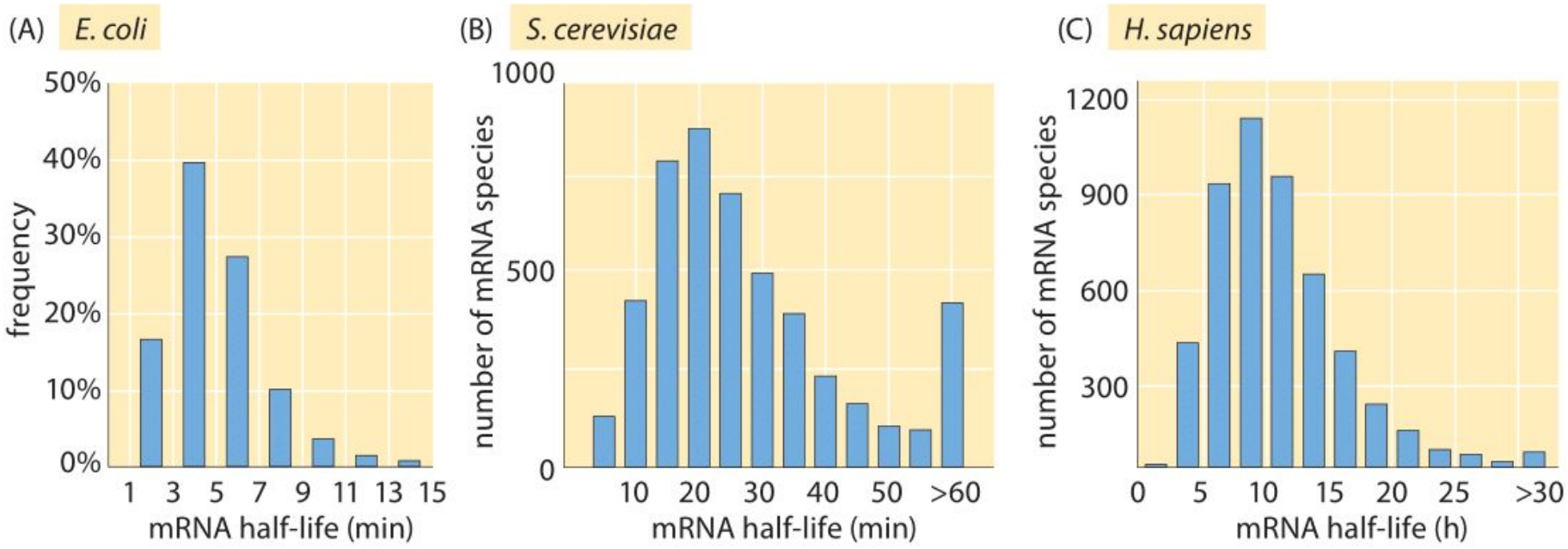
$$\frac{d\overline{m}}{dt} = r - \gamma\overline{m}$$

where r is the rate of mRNA production and γ is the rate constant dictating mRNA decay.

The steady-state value of the mRNA is given by

$$\overline{m} = \frac{r}{\gamma}$$

showing that to first approximation, it is the balance of the processes of production and decay that controls the steady-state levels of these molecules. If our equation is for the copy number of molecules per cell, there will be an abrupt change in the number each time the cells divide since the total mRNA and protein content is partitioned between the two daughter cells.



The lifetime of mRNA molecules is usually short in comparison with the fundamental time scale of cell biology defined by the time between cell divisions. As shown in Figure 1A, for E. coli, the majority of mRNA molecules have lifetimes between 3 and 8 minutes.

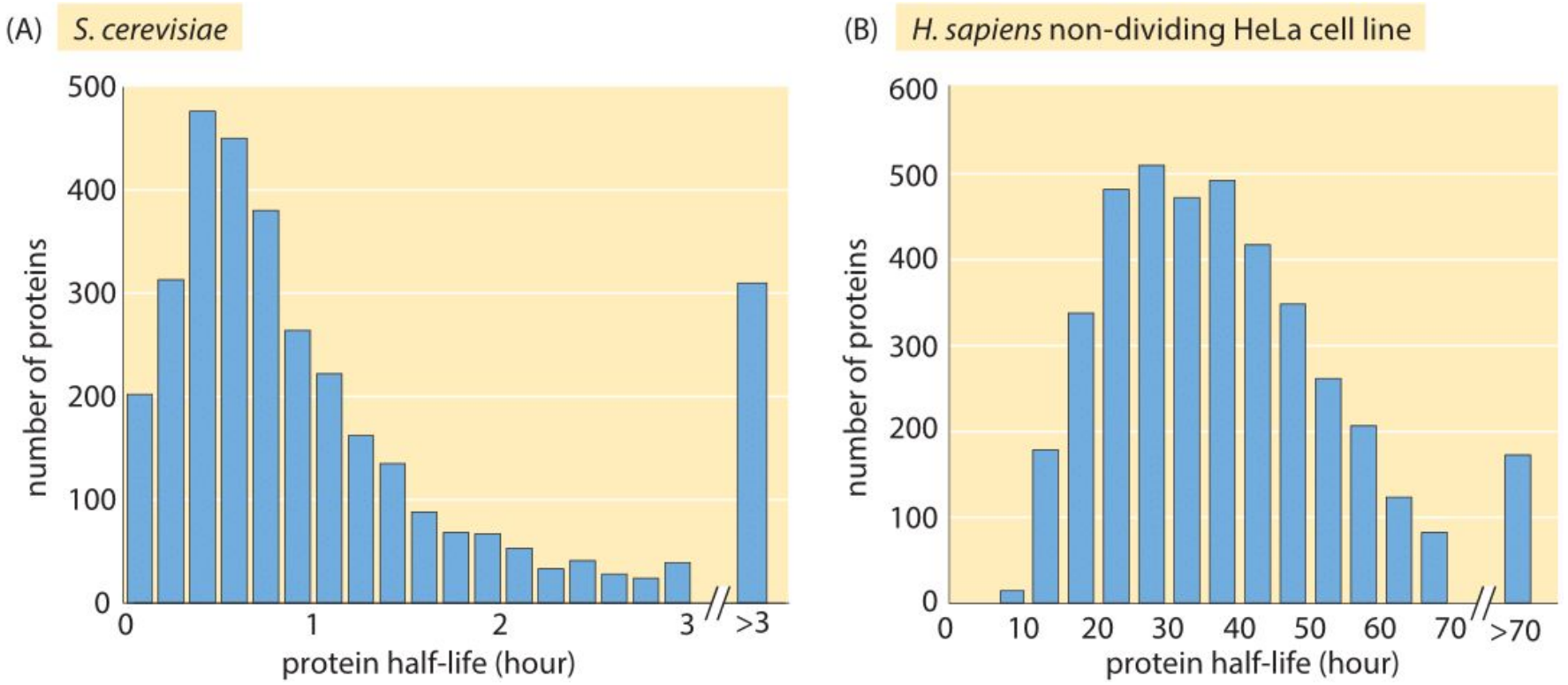


Figure 3: Measured half lives of proteins in budding yeast and a HeLa human cancer cell line. The yeast experiment used the translation inhibitor cycloheximide which disrupts normal cell physiology. The median half life of the 4100 proteins measured in the non-dividing HeLa cell is 36 hours. (A, adapted from A. Belle et al., Proc. Natl Acad. Sci. USA 103:13004, 2006; B, adapted from S. Cambridge et al, J. Proteome Res. 10:5275, 2011.)

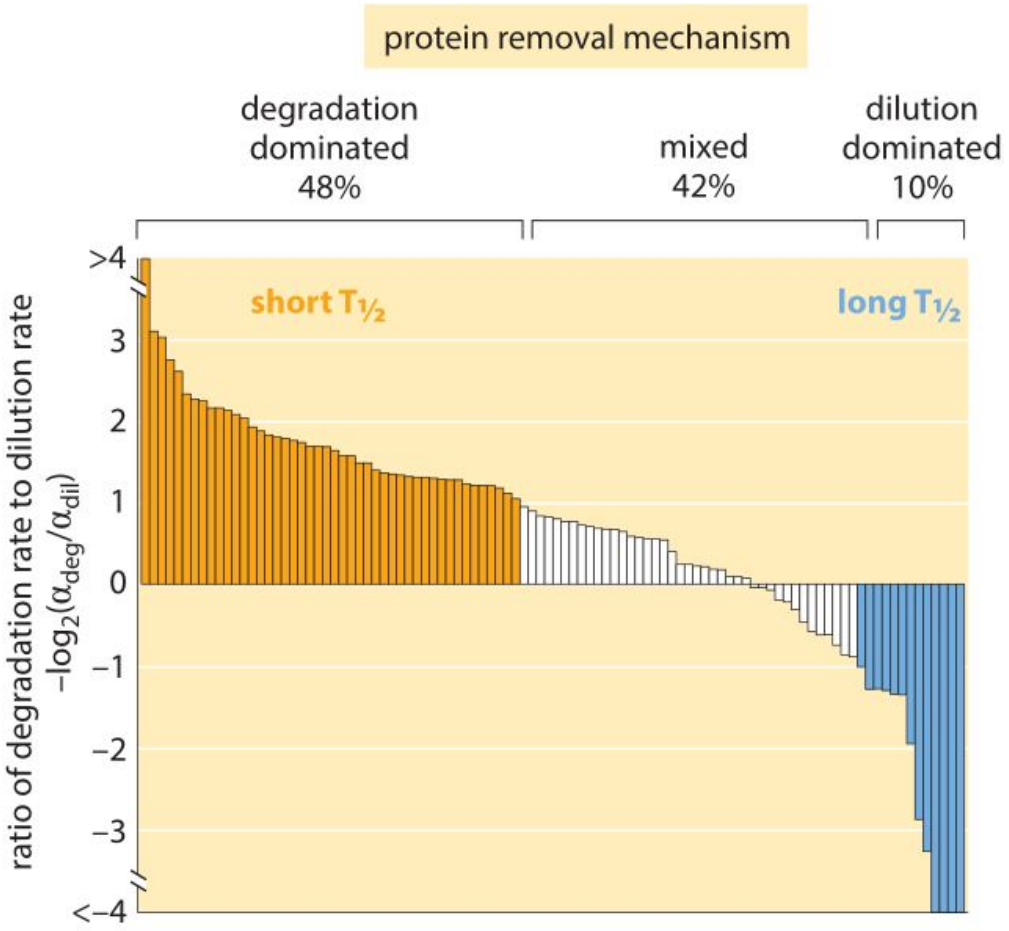


Figure 4: Distribution of 100 proteins from a H1299 human cell line, comparing the rate of degradation to dilution to find which removal mechanism is dominant for each of the proteins. The overall removal rate alpha ranges between 0.03 and 0.82 hour-1 with an average of 0.1+/-0.09 hour-1. This is equivalent to half life of ≈7 hours via the relationship half-life, T1/2 = ln(2)/alpha. Adapted from E. Eden et al, Science, 331:764, 2011.

CELL LIFE CYCLE

HOW LONG DOES IT TAKE CELLS TO COPY THEIR GENOMES?

But what sets the time scale for the replication process itself and how do the mechanisms and associated rates differ from one organism to the next?

Does the time required to complete replication ever impose a limitation on the growth rate of the organism?

In order directly measure the replication rate a single-molecule technique in which the progress of the replication machinery is monitored by using a microscope to watch the motion of a tiny bead attached to the DNA template is used.

By permitting only leading-strand synthesis, the replication process results in the conversion of double-stranded DNA into one double-stranded fragment and a second single-stranded fragment on the uncopied strand.

The method exploits the difference in entropic elasticity of the single-stranded and double-stranded fragments.

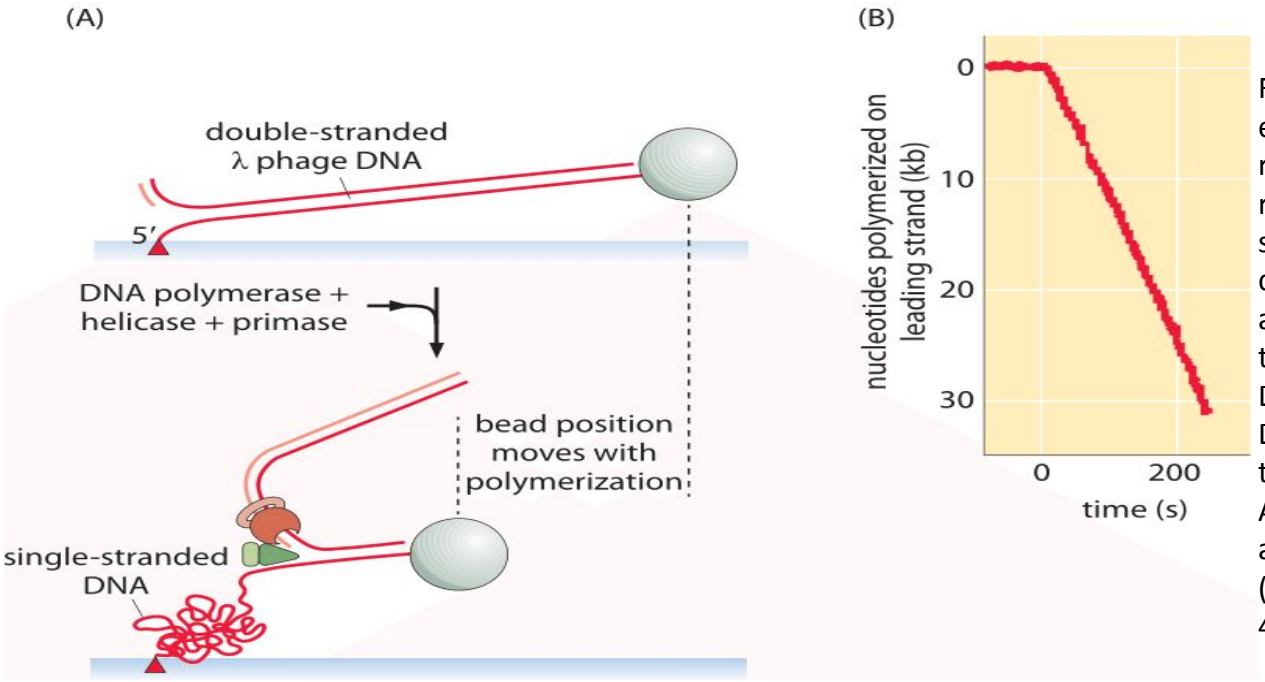


Figure 1: Schematic of single-molecule experiment used to measure the rate of replication. (A) The progress of the replication process performs leading strand synthesis and thus converts double-stranded into single-stranded (plus another double stranded) DNA. (B) Because the "spring constant" of single-stranded DNA is larger than that for double-stranded DNA, the stretched tether recoils resulting in the bead position time course shown. Adapted from: Lee et al, DNA primase acts as a molecular brake in DNA replication. (Adapted from J.-B. Lee et al., Nature. 439:621, 2006.)

As a result, with increasing replication, more of the template is converted into the single-stranded form which as seen in Figure 1 serves as a much stronger entropic spring than the double-stranded fragment whose persistence length is orders of magnitude larger. The spring moves the bead at the same rate as the polymerase proceeds forward, serving as a readout of the underlying replication dynamics. **These measurements resulted in an in vitro replication rate of 220 ± 80 nucleotides/s for the replication machinery from a T7 bacterial virus.**

With a genome size of ≈40,000 bp and without taking into account initiation and similar processes that might complicate directly importing these in vitro insights to the in vivo setting, we can estimate that it will require at least **40,000 bp/220 bp/s ≈200 s or about 3 minutes to replicate the compact viral genome.**

E. coli has a genome of roughly 5 million bp.

Replication rates are observed to be several hundred bp/sec

Replication in bacteria takes place with *two replication forks heading in opposite directions* around the circular bacterial chromosome.

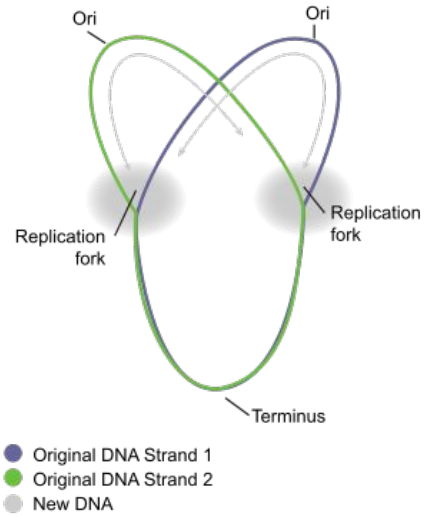
The replication rates imply that it should take the two replisomes at least 2500 sec (≈40 minutes) to replicate the genome, a number that is much longer than the minimal division time of ≈20 minutes

This interesting estimate delivers a paradox that is resolved by the observation that E. coli under ideal growth conditions employs *nested replication forks*

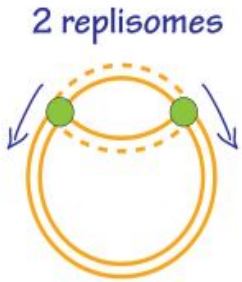
At fast growth rates more than 6 origins of replication and over 10 replication forks coexist in a single cell

Recently, single-molecule microscopy revealed that the most common stoichiometry of the replication machinery, the replisome, consists of 3 DNA polymerases per replisome in contrast to the naïve picture of 2 DNA polymerases. It seems that the third polymerase can sometimes be engaged in the lagging strand replication together with another polymerase or in other cases to be awaiting engagement in the replication process.

Doubling time (min)	Number of origins of replication per cell	Number of replication forks per cell
24	6.5	9.2
30	4.7	5.9
40	3.4	3.6
60	2.4	2.1
100	2.0	1.5



genome replication paradox



genome length $\approx 5 \times 10^6$ bp

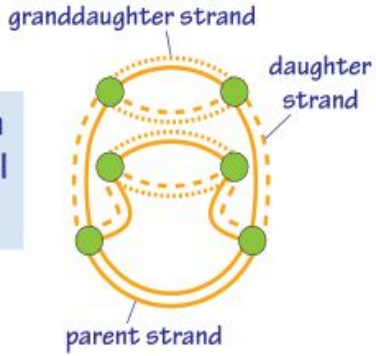
replication rate $\leq$ 1000 bp/s per replisome

$$\tau^{\text{minimum}}_{\text{predicted}} = \frac{5 \times 10^6 \text{ bp}}{1000 \frac{\text{bp}}{\text{s} \times \text{replisome}} \times 2 \text{ replisomes}} \approx 2500 \text{ s} \approx 40 \text{ min}$$

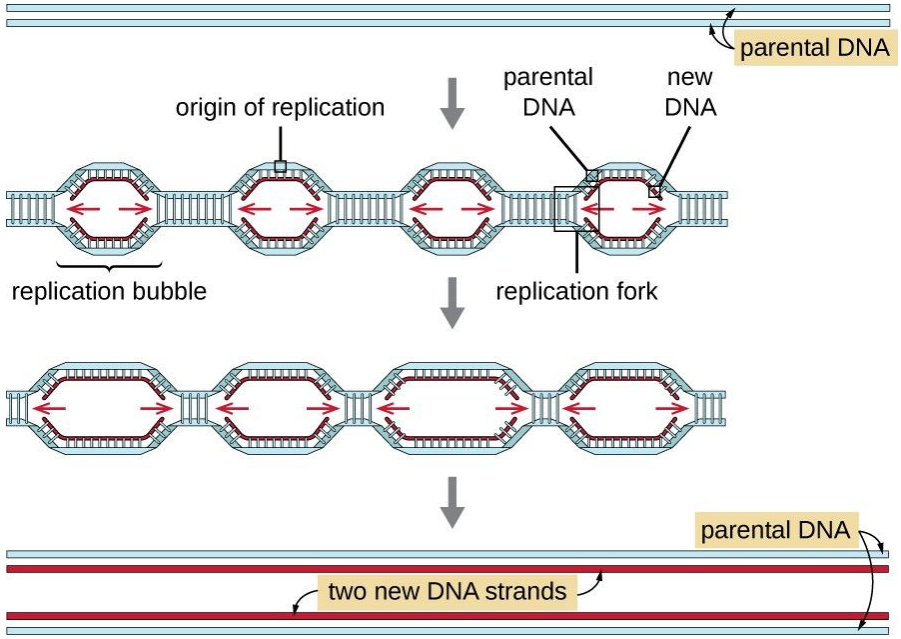
$\tau^{\text{minimum}}_{\text{predicted}}$: doubling time

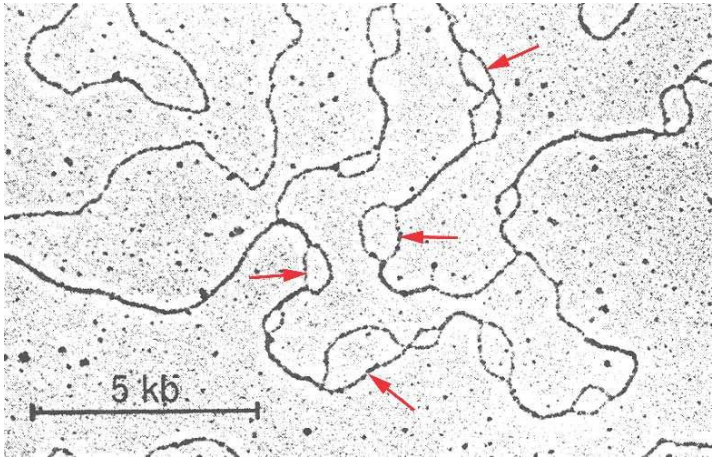
longer than $\tau^{\text{minimum}}_{\text{measured}} \approx 20$ min

$\Rightarrow$ nested replication forks are essential for max growth



Nested replication forks. The schematic shows the way in which multiple rounds of replication are taking place simultaneously in rapidly dividing E. coli cells. This picture is used to make an estimate of the time to replicate the full bacterial genome. Recent measurements using fluorescently tagged components of the replication machinery reported values of 55-65 minutes for DNA replication suggesting an in vivo average **replication rate of about 600 bp/s .**





https://wou.edu/chemistry/courses/online-chemistry-textbooks/ch450-and-ch451-biochemistry-defining-life-at-the-molecular-level/chapter-9-dna-replication-and-repair-2/#9.4eukar

Eukaryotic genomes are usually much larger than those of their prokaryotic cousins and as a result, the replication process must depend upon more than a single origin of replication.

The number of origins leading to replication is a subject of active research recently using microarrays and deep sequencing to find peaks of DNA content in S-phase indicating putative origins.

In mouse the number of origins vary from as low as 1,000 to as many as 100,000, while for Drosophila the estimate is about 10,000.

Each origin is associated with a replisome that proceeds at a rate of 4-40 bp/s or roughly 1 kb/min

For rapid genome replication in the early stage of D. melanogaster development where the embryo replicates its ≈120 million bp genome pace of once **every ≈8 minutes**

In humans the S phase (synthesis phase) in many cell types is on the order of **10 hours**

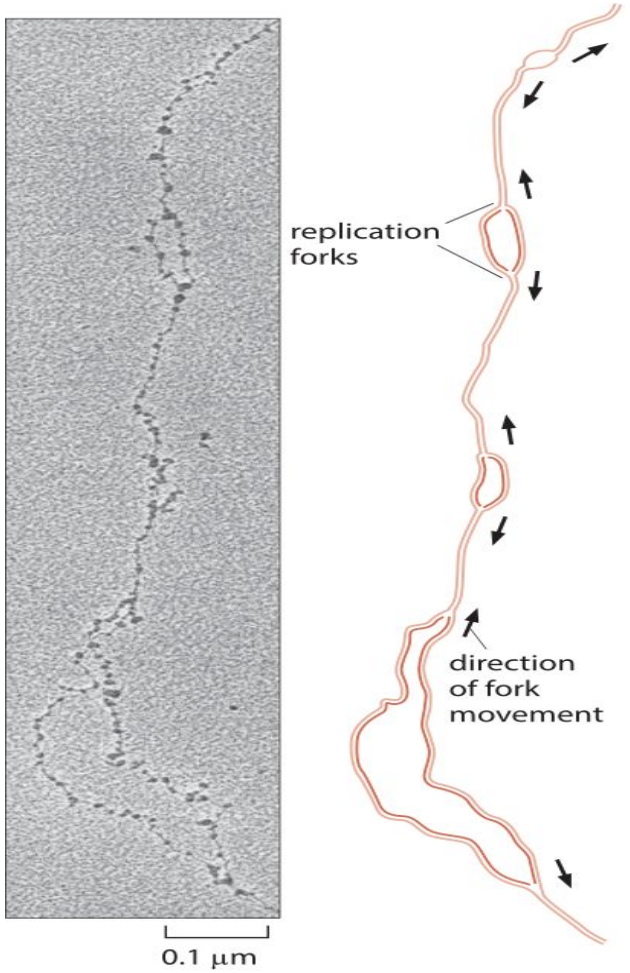


Figure 3: Replication forks in D. melanogaster. Replication forks move away in both directions from replication origins. (Electron micrograph courtesy of Victoria Foe. Adapted from B. Alberts et al., Molecular Biology of the Cell, 5th ed. New York, Garland Science, 2008.)

HOW LONG DO THE DIFFERENT STAGES OF THE CELL CYCLE TAKE?

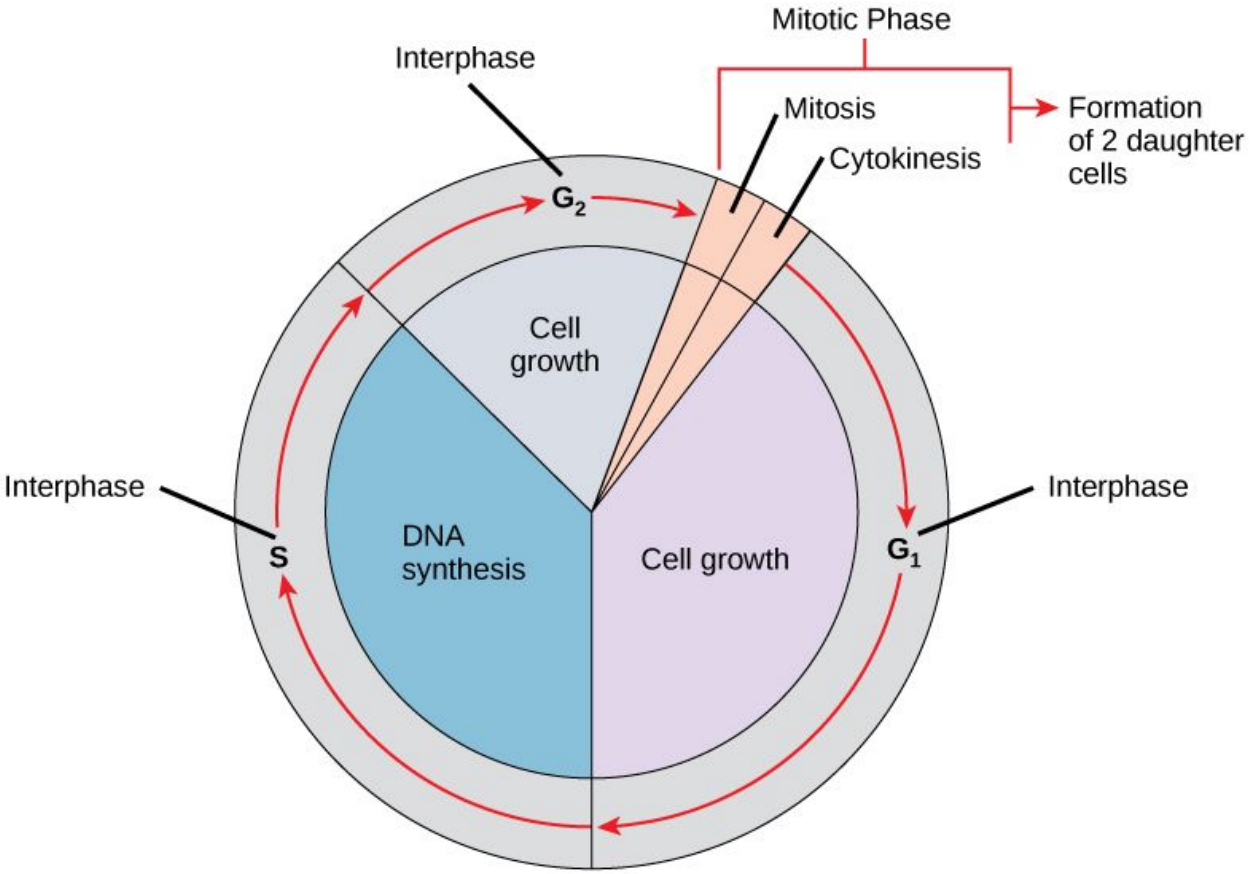
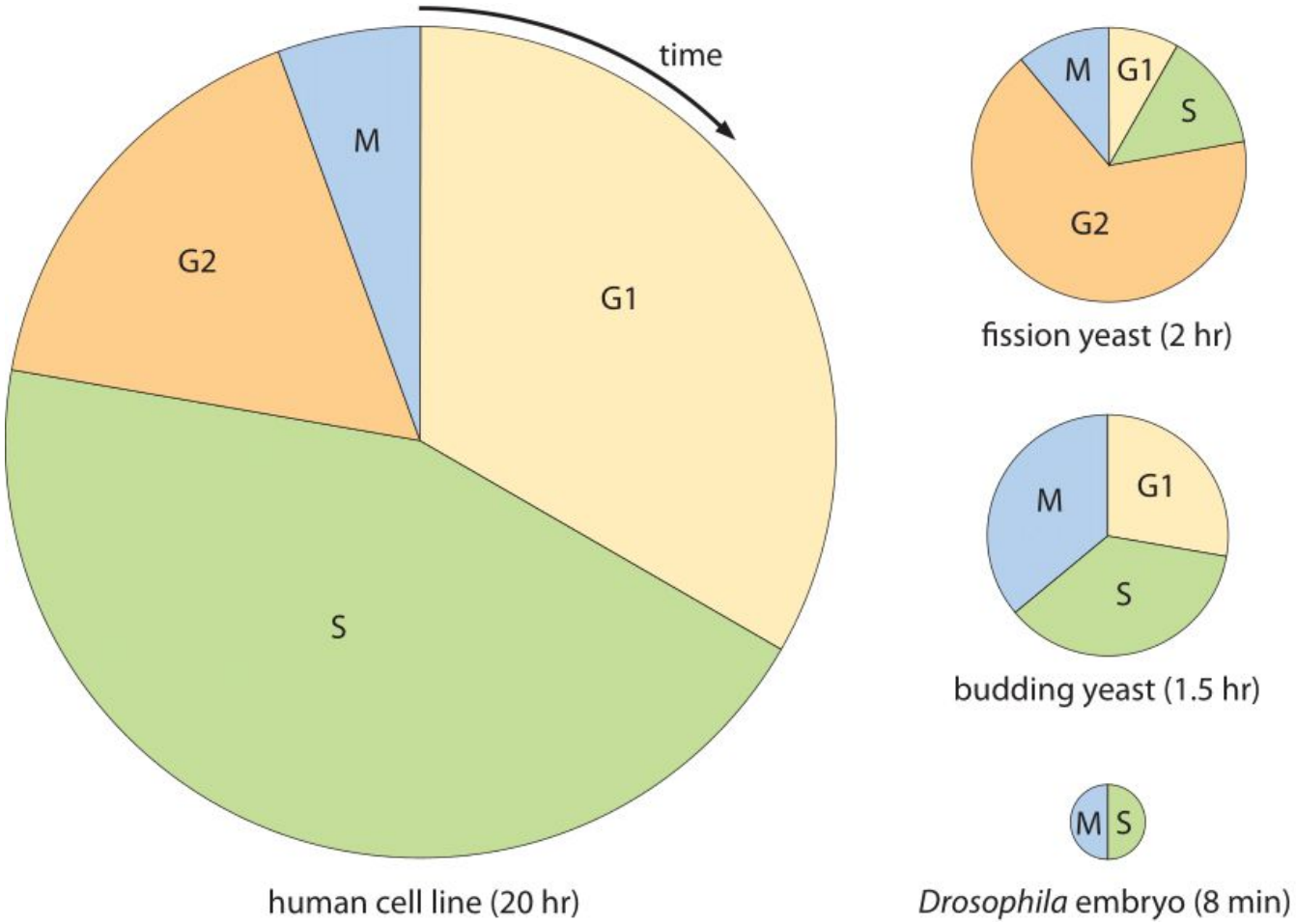


Diagram of the Cell Cycle. A cell moves through a series of phases in an orderly manner. During interphase, G1 involves cell growth and protein synthesis, the S phase involves DNA replication and the replication of the centrosome, and G2 involves further growth and protein synthesis. The mitotic phase follows interphase. Mitosis is nuclear division during which duplicated chromosomes are segregated and distributed into daughter nuclei. Usually the cell will divide after mitosis in a process called cytokinesis in which the cytoplasm is divided and two daughter cells are formed.

https://wou.edu/chemistry/courses/online-chemistry-textbooks/ch450-and-ch451



Cell cycle times for different cell types. Each pie chart shows the fraction of the cell cycle devoted to each of the primary stages of the cell cycle. The area of each chart is proportional to the overall cell cycle duration. Cell cycle durations reflect minimal doubling times under ideal conditions. (Adapted from “The Cell Cycle – Principles of Control” by David Morgan.)

HOW QUICKLY DO DIFFERENT CELLS IN THE BODY REPLACE THEMSELVES?

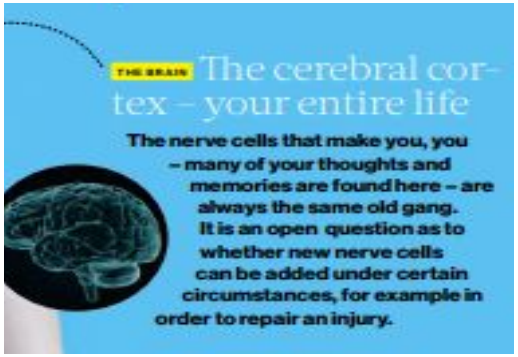
cell type	turnover time	BNID
small intestine epithelium	2-4 days	107812, 109231
stomach	2-9 days	101940
blood Neutrophils	1-5 days	101940
white blood cells Eosinophils	2-5 days	109901, 109902
gastrointestinal colon crypt cells	3-4 days	107812
cervix	6 days	110321
lungs alveoli	8 days	101940
tongue taste buds (rat)	10 days	111427
platelets	10 days	111407,111408
bone osteoclasts	2 weeks	109906
intestine Paneth cells	20 days	107812
skin epidermis cells	10-30 days	109214, 109215
pancreas beta cells (rat)	20-50 days	109228
blood B cells (mouse)	4-7 weeks	107910
trachea	1-2 months	101940
hematopoietic stem cells	2 months	109232
sperm (male gametes)	2 months	110319, 110320
bone osteoblasts	3 months	109907
red blood cells	4 months	101706, 107875
liver hepatocyte cells	0.5-1 year	109233
fat cells	8 years	103455
cardiomyocytes	0.5-10% per year	107076, 107077, 107078
central nervous system	life time	101940
skeleton	10% per year	109908
lens cells	life time	109840
oocytes (female gametes)	life time	111451

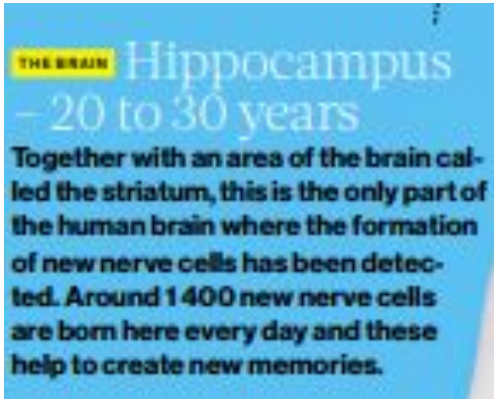
How can the replacement rates of the cells in various tissues in our body be measured?

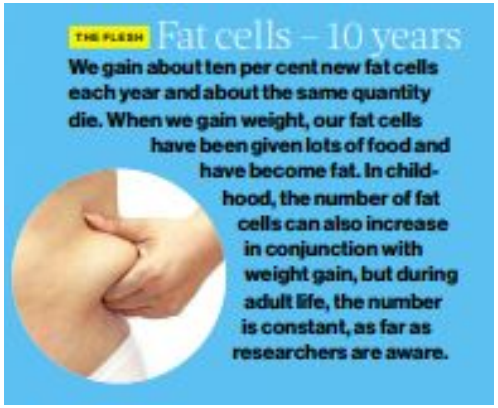
The level of 14C in genomic DNA closely parallels atmospheric levels and can be used to establish the time point when the DNA was synthesized and cells were born.

Fat cells (adipocytes) replace at a rate of 8±6% per year. This results in the replacement of half of the body's adipocytes in ≈8 years.

Replacement of heart muscle cells occurs albeit at a slow rate. Estimates vary from 0.5% per year to as high as 30% per year depending on age and gender.







Alterations in cell turnover are a key feature in several diseases-

- decreased erythrocyte production - aplastic anemia
- increased keratinocyte turnover- psoriasis
- decreased neurogenesis - depression
- impaired production of cardiomyocytes - heart failure

Inspired by 14C-dating in archeology Spalding et.al 2005 developed a way to retrospectively determine the age of cells without the need for delivering any chemical to the individual prior to the analysis.
14C levels on earth have remained relatively constant over long time periods, and the radioactive decay of the isotope is used to retrospectively date biological material in archeology. The resolution in modern time is poor due to a half-life of 5730 years.

14C in Genomic DNA Reflects the Age of Cells.

Most molecules in a cell are in constant flux, with the unique exception of genomic DNA, which is not exchanged after a cell has gone through **its last division**. The level of 14C integrated into genomic DNA should thus reflect the level in the atmosphere at any given time point.
Determination of 14C levels in genomic DNA could be used to retrospectively establish the birth date of cells in the human body.

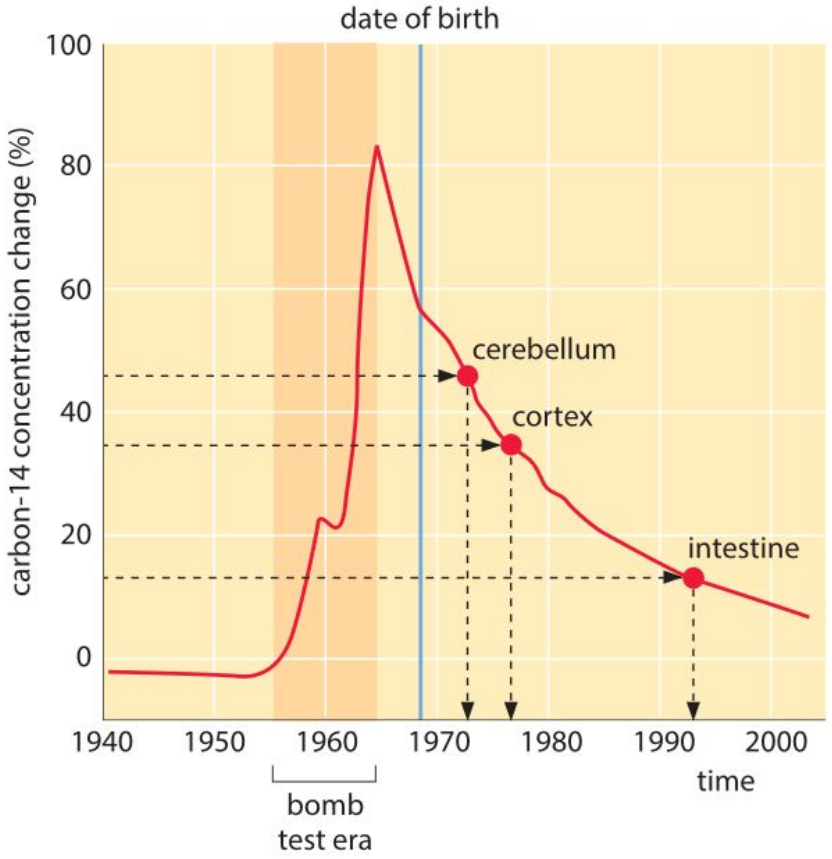
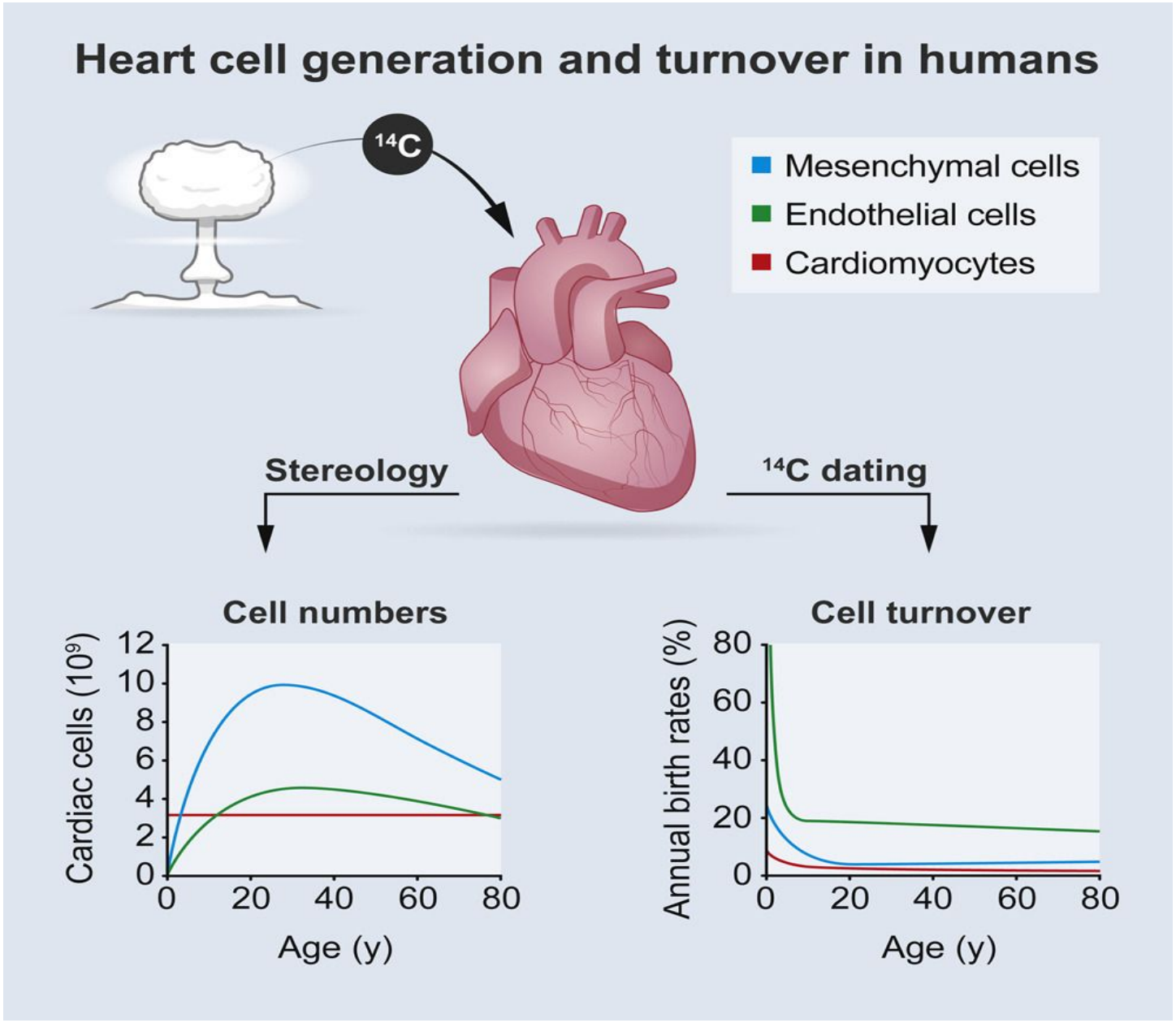


Figure 1. Inferring tissue turnover time from natural stable isotope labeling. The global 14C Levels in the environment are shown in red. A large addition of 14C in 1955–1963 is the result of nuclear bomb tests. Cell age in different adult human organs is inferred from analysis of 14C levels in genomic DNA measured in 2003-4 from the cerebellum, occipital-cortex, and small intestine. Birth year of the individual is indicated by a vertical line. Stable isotope levels reveal the differing turnover rates of cells in different tissues. (Adapted from K. L. Spalding, et al., Cell, 122:133-143, 2005.)


Heart cell generation and turnover in humans
14C
Mesenchymal cells
Endothelial cells
Cardiomyocytes
Stereology
14C dating
Cell numbers
Cardiac cells (10^9)
12
10
8
6
4
2
0
0
20
40
60
80
Age (y)
Cell turnover
Annual birth rates (%)
80
60
40
20
0
0
20
40
60
80
Age (y)