BE/APH161 – PHYSICAL BIOLOGY OF THE CELL

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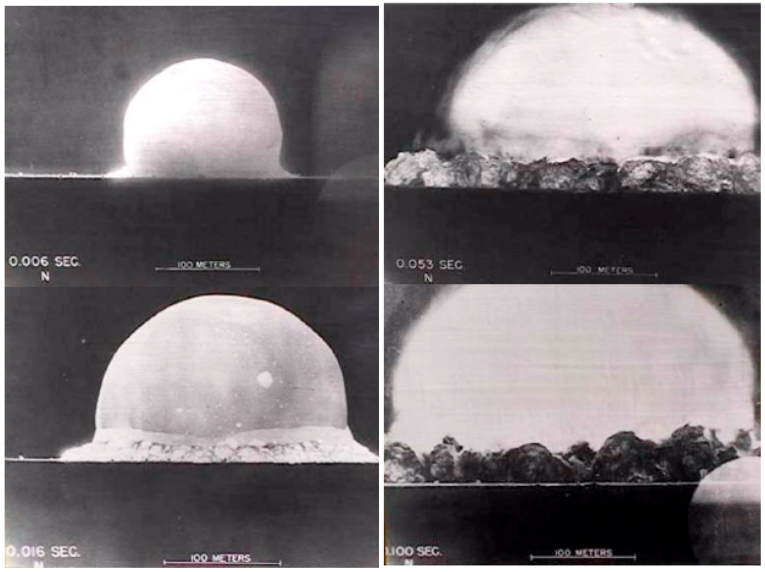
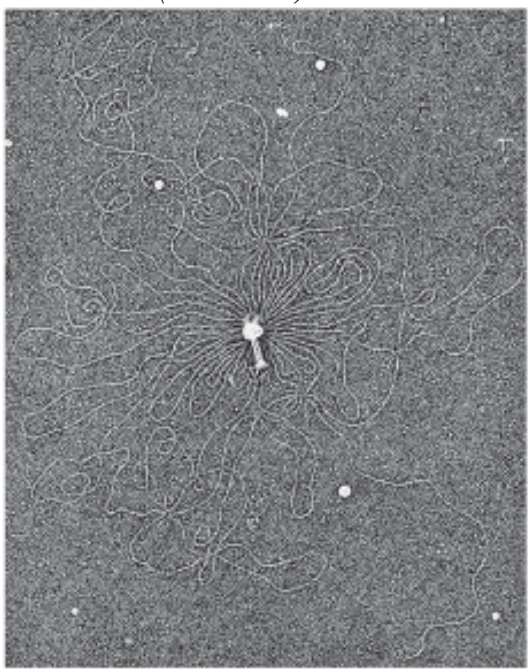
First topic
Gnome management: a feeling for the numbers

THE POWER OF ESTIMATION: AN ANALOGY

- ***Politicians and generals can make some information "classified" and it can be circumvented by cleverness.***
- ***Taylor made simplifying assumptions such as "spherical" blast.***
- ***This is a segue into our main topic: genomes and their use. Estimates on genome management.***
- ***Same idea could be used to estimate genome length.***
- ***The concept: figure out the length of the genome using a single picture and pure thought!***



(G. Stent)



ESTIMATIONS ON GENOME MANAGEMENT: HOW BIG ARE GENOMES?

- ***Use the simplest nunchuk physics of random walks to estimate the genome size.***
- ***What makes DNA different from some other polymer? The persistence length!***
- ***The radius of gyration scales as $N^{1/2}$, which allows us to estimate the number of such Kuhn segments and hence back out the genome length.***
- ***Note: This also tells us that work needs to be done to squish genomes into their hosts.***

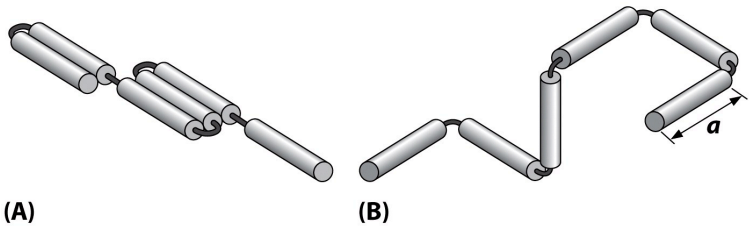
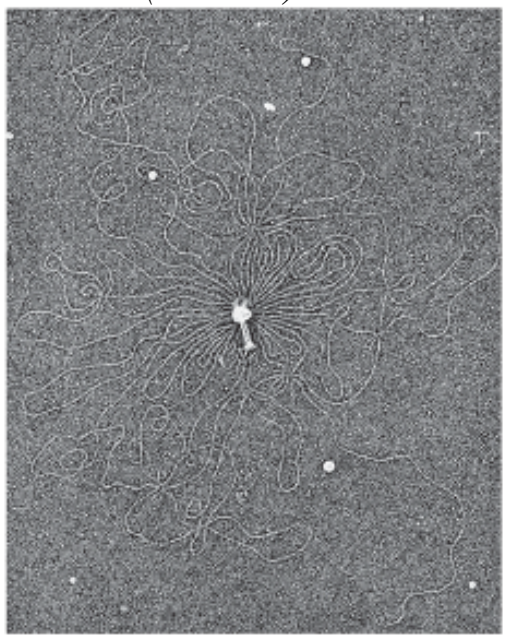


Figure 8.1 Physical Biology of the Cell (© Garland Science 2009)

(G. Stent)



(R. Kavenoff)

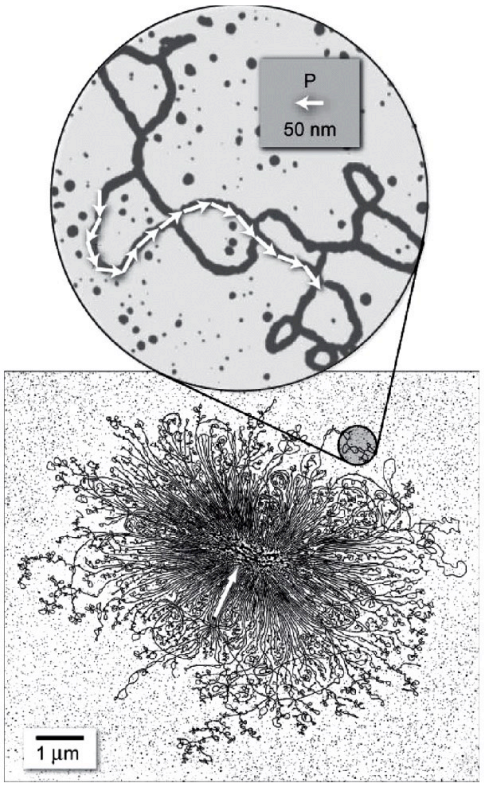
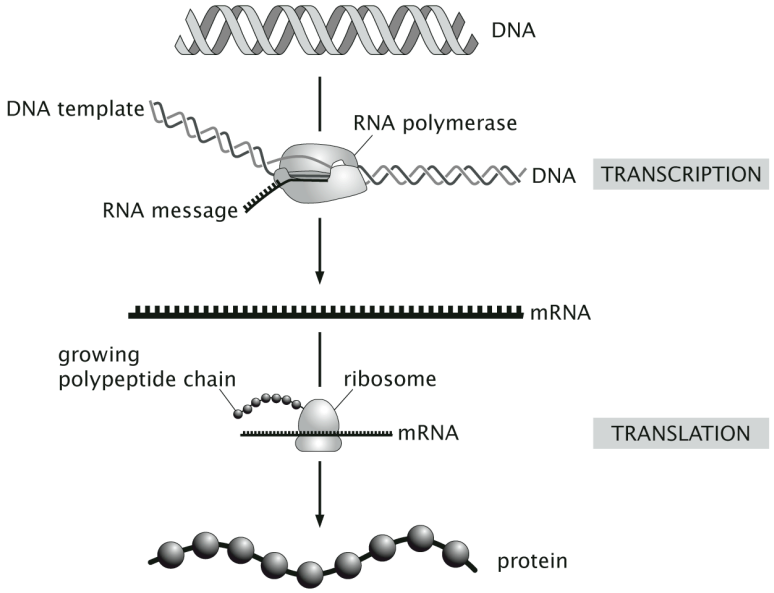


Figure 8.6 Physical Biology of the Cell (© Garland Science 2009)

PUTTING GENOMES TO WORK: THE CENTRAL DOGMA

- ***Francis Crick referred to nucleic acids and proteins as "the two great polymer languages".***
- ***One of the great stories of modern biology is the working out of the mechanisms behind the way information read out in one polymer language (nucleic acids) is converted into information in the second polymer language (proteins).***
- ***A beautiful and key insight: the genetic code.***



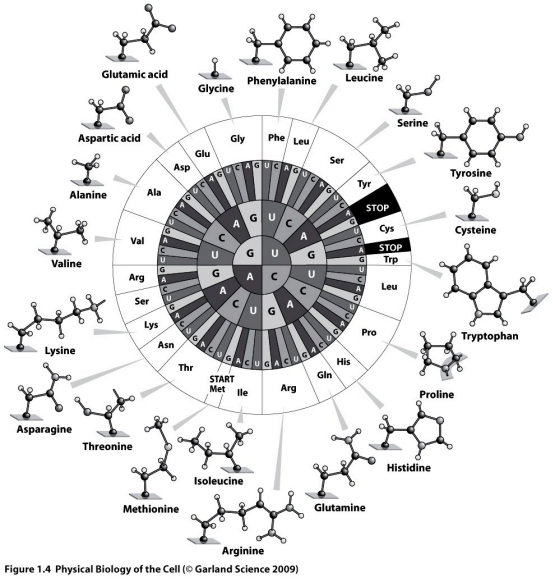


Figure 1.4 Physical Biology of the Cell (© Garland Science 2009)

THINKING UP THE NUMBER OF GENES: "WHAT IS TRUE FOR E. COLI IS TRUE FOR THE ELEPHANT"

- ***A betting pool (Las Vegas for estimates) was set up on the number of genes in the human genome and responses varied from 25,000 to 150,000.***
- ***A winner was declared, but the issue remains unsettled.***
- ***Simplest logic: use "typical" protein size of 300 amino acids, which requires roughly 1000 nucleotides to code for them. This naïve estimate says:***

$$N_{genes} \approx N_{bp} / 1000$$

- ***Works great for E. coli, fails miserably for humans (and elephants).***

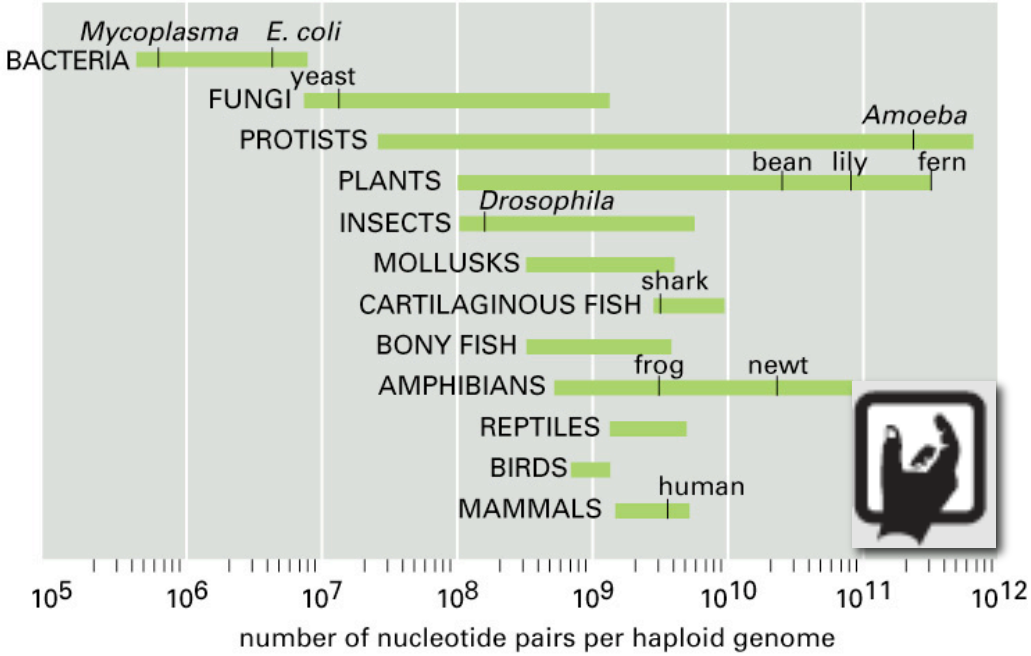


Figure 1–38. Molecular Biology of the Cell, 4th Edition.

The bet: genesweep pool- Science 6 June 2003:

Vol. 300. no. 5625, p. 1484

A Low Number Wins the GeneSweep Pool

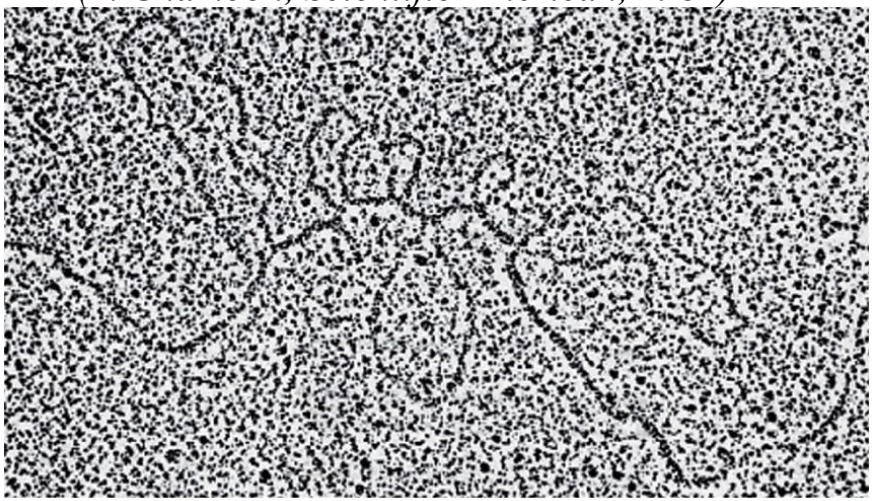
Elizabeth Pennisi

COLD SPRING HARBOR, NEW YORK--The human genome has been sequenced, but calculating the number of genes it contains is taking more time. DNA experts have nonetheless decided they know who made the best prediction.

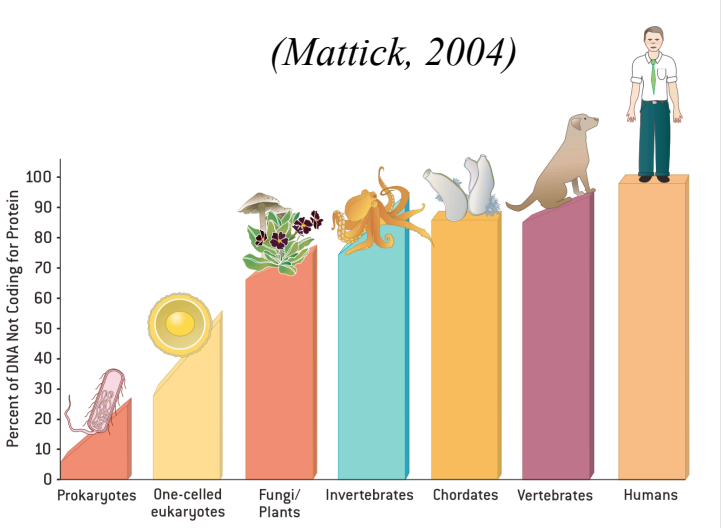
THE PROBLEM WITH EUKARYOTES

- ***Conundrums and surprises are a great way to learn things. The failure of the simplest estimate for eukaryotes reveals the important concept of "split genes", referring to the fact that the coding regions are riddled with "introns".***
- ***The failure of our estimate also reflects the existence of endogenous retroviruses and all sorts of other interesting genomic stories.***

(P. Chambon, Scientific American, 1981)



(Mattick, 2004)



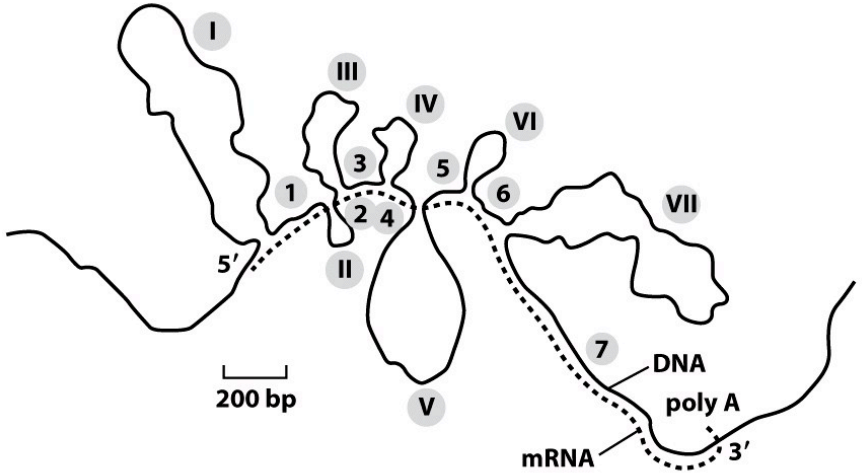


Figure 4.28 Physical Biology of the Cell (© Garland Science 2009)

SECOND TOPIC
HOW FAST DO BIOLOGICAL PROCESSES OCCUR

THE RATES OF THINGS IN BIOLOGY: A FEELING FOR THE NUMBERS

- Bio structures exist over a wide range of spatial scales…

  …and bio processes take place over time scales from < ns to the age of the Earth.

- A standard stopwatch: the cell cycle of *E.coli*
- Cells strategy:

 manage time by stringing together processes in succession;

 manipulate time (*e.g., using enzymes*) to alter the intrinsic rates of processes

- To some extent, coupling btw temporal and spatial scales (*small things tend to operate at faster rates…*)

THE STANDARD STOPWATCH

bacterial cell division

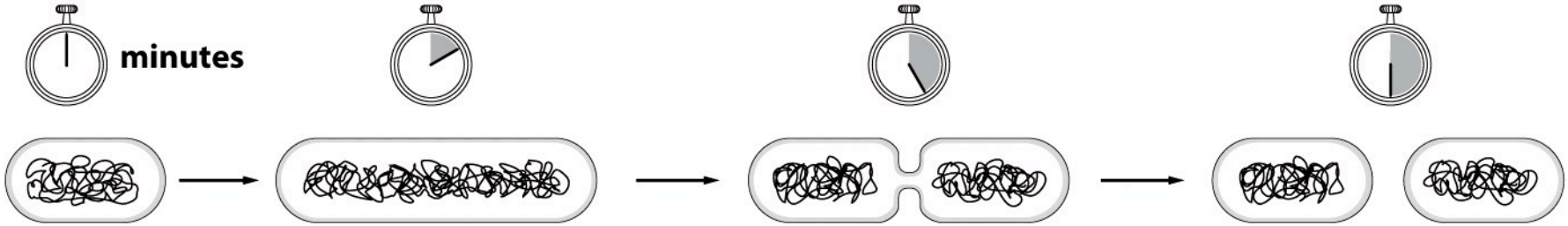
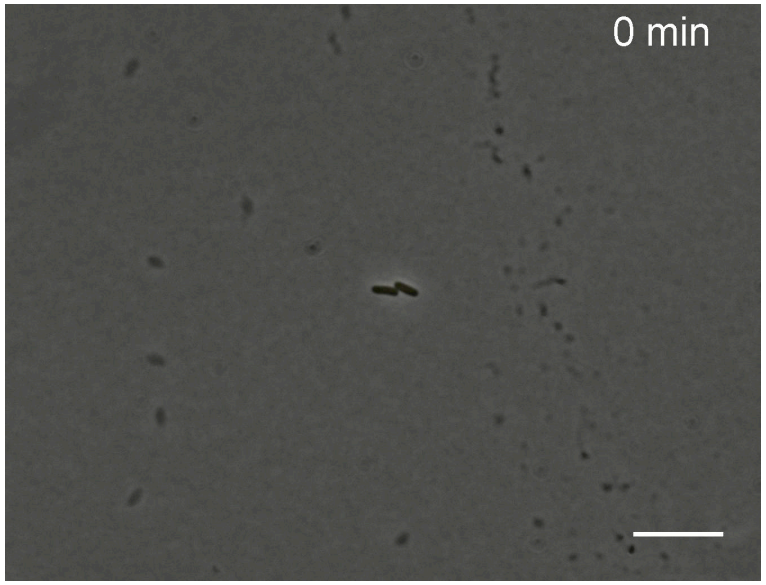


Figure 3.2c Physical Biology of the Cell (© Garland Science 2009)

- *Cell cycle = set of processes whereby a single cell becomes 2 daughter cells, through the process of cell division*

(courtesy of Linda Song)



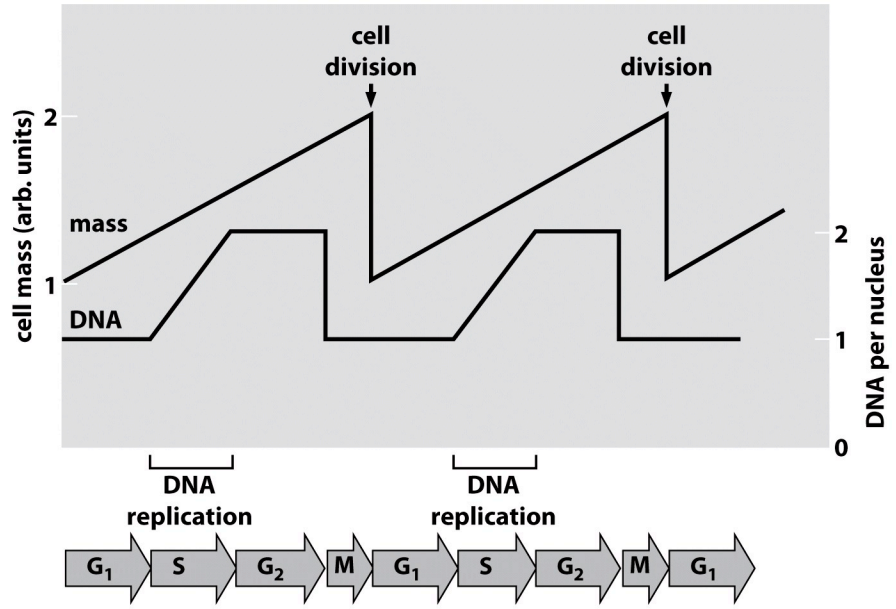
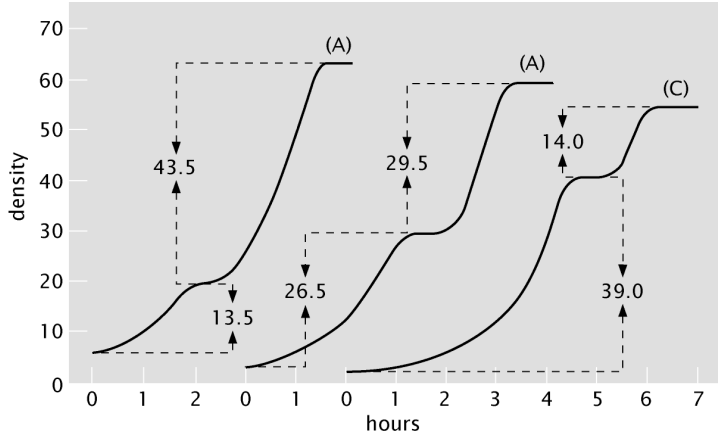


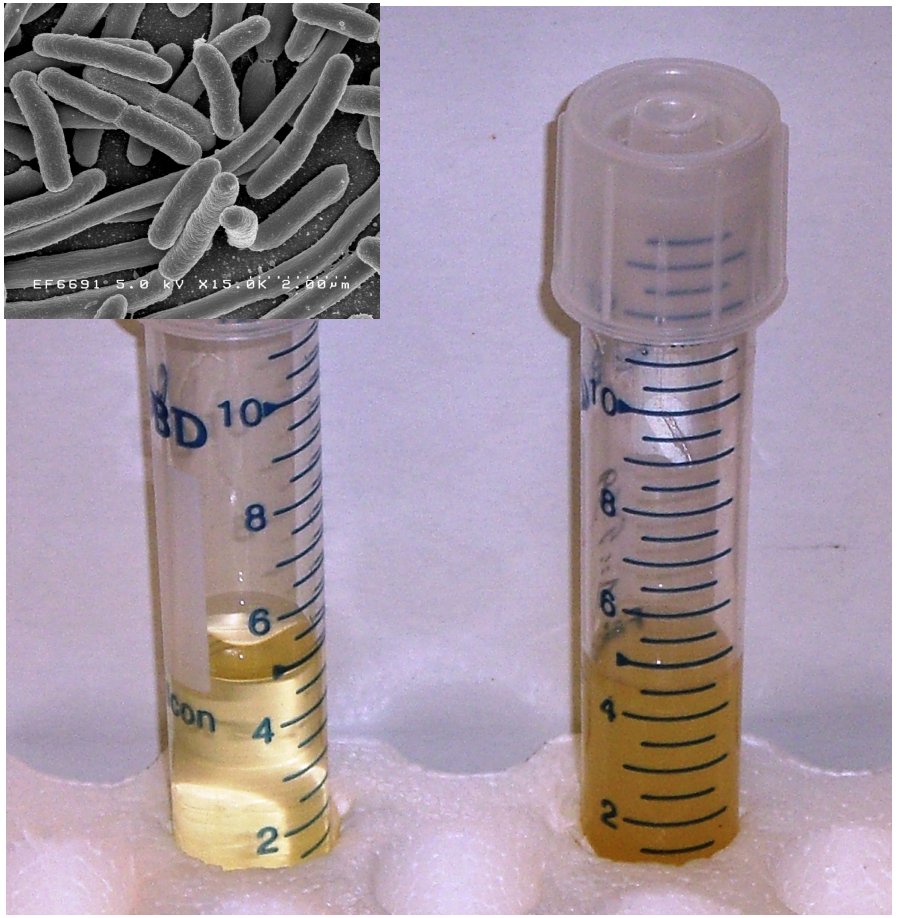
Figure 3.15b Physical Biology of the Cell (© Garland Science 2009)

GROWTH CURVES REVEAL CELLS CARE WHAT'S FOR DINNER

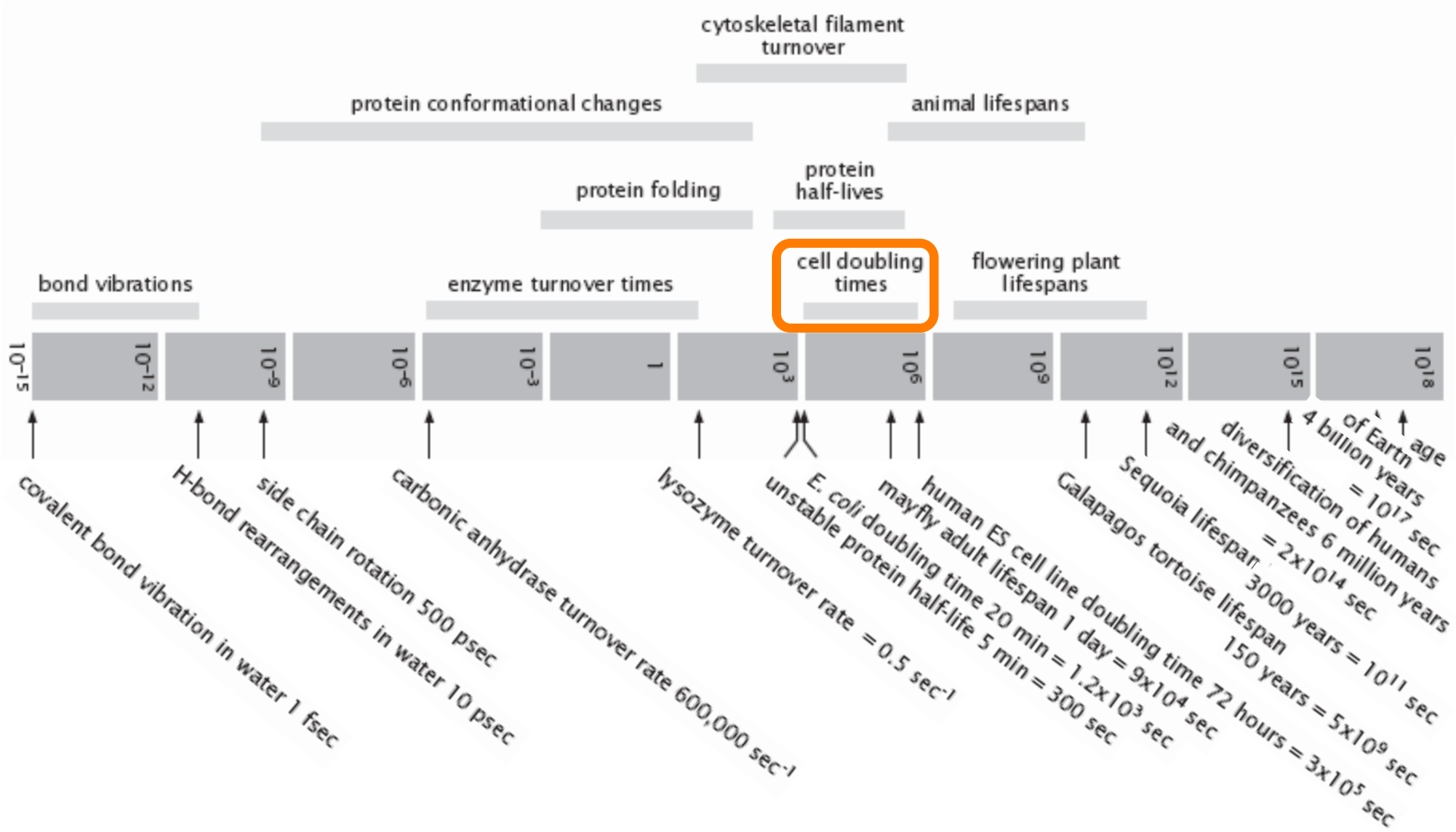
- ***The fundamental mystery of life: Take 5 mL of solution with some salt and sugar, add a single cell, and 12 hours later you will have 10^{10} cells!***
- ***These growth curves tell the story of how genes are turned on and off in response to environmental cues.***



Bacterial growth curves

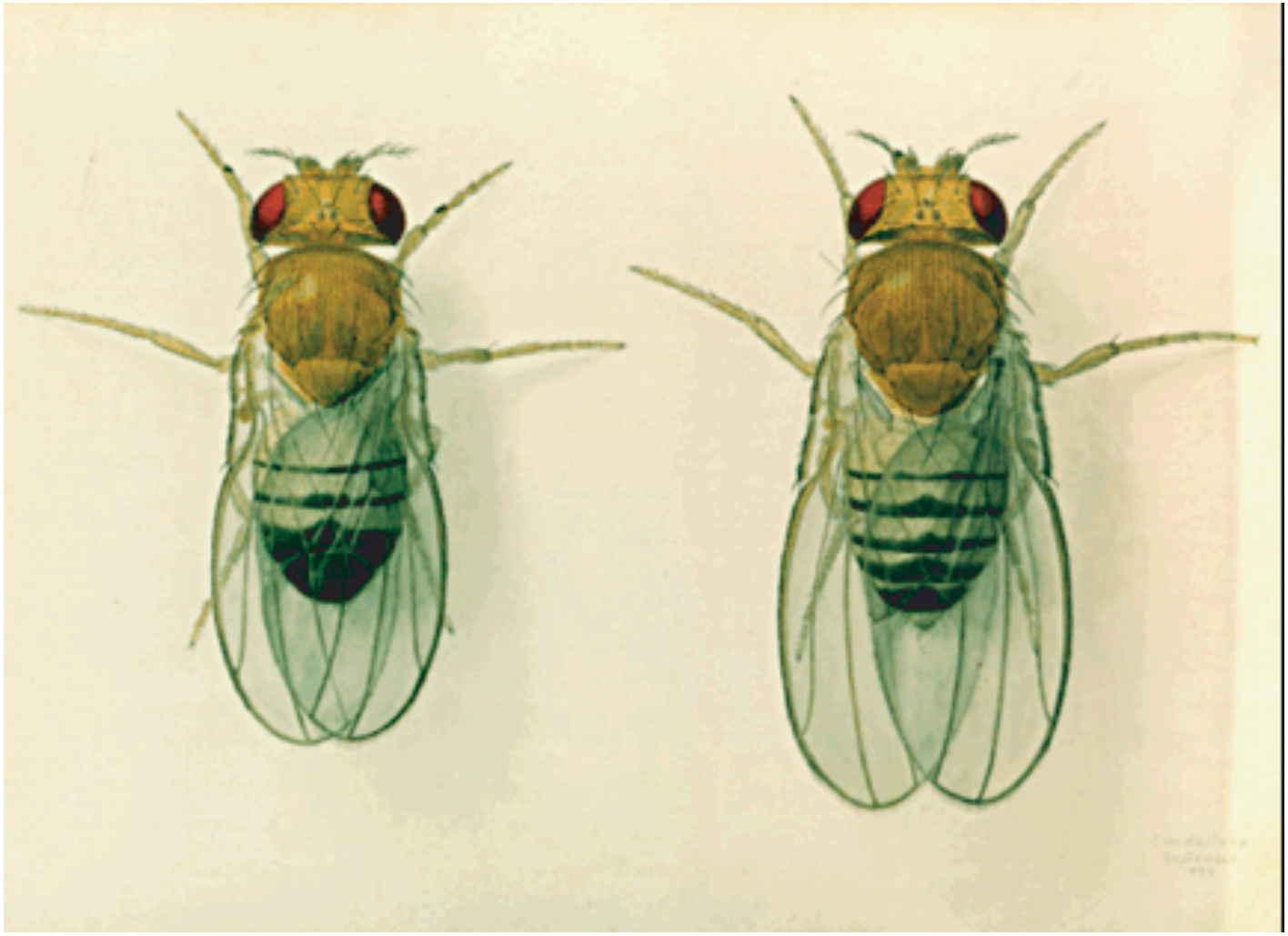


THE HIERARCHY OF TEMPORAL SCALES

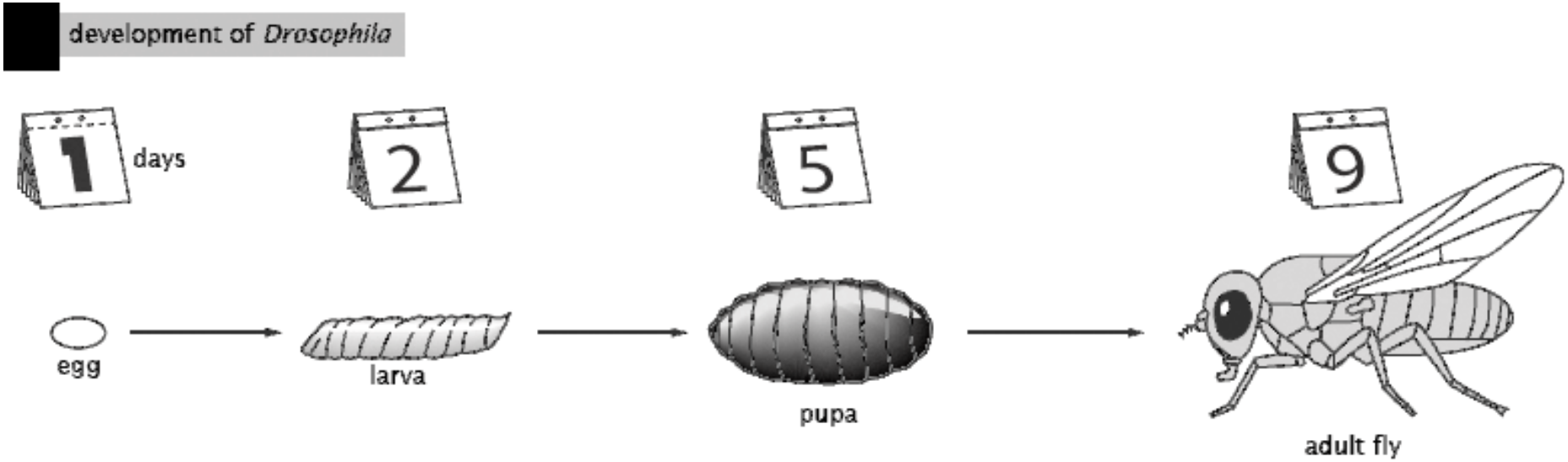


Molecular motion of biochem. species as they interact and change identity → *Unfolding of the lives of individual cells* → *Trajectories of entire species*

TO BUILD A FLY



BIOLOGICAL TIME SCALES IN POWERS OF 10: 10 DAYS



- *Drosophila melanogaster: a workhorse of developmental biology*
- *~ 10 days btw fertilization of the egg and the emergence of a fully functioning adult fly*

BIOLOGICAL TIME SCALES IN POWERS OF 10: 10 HOURS

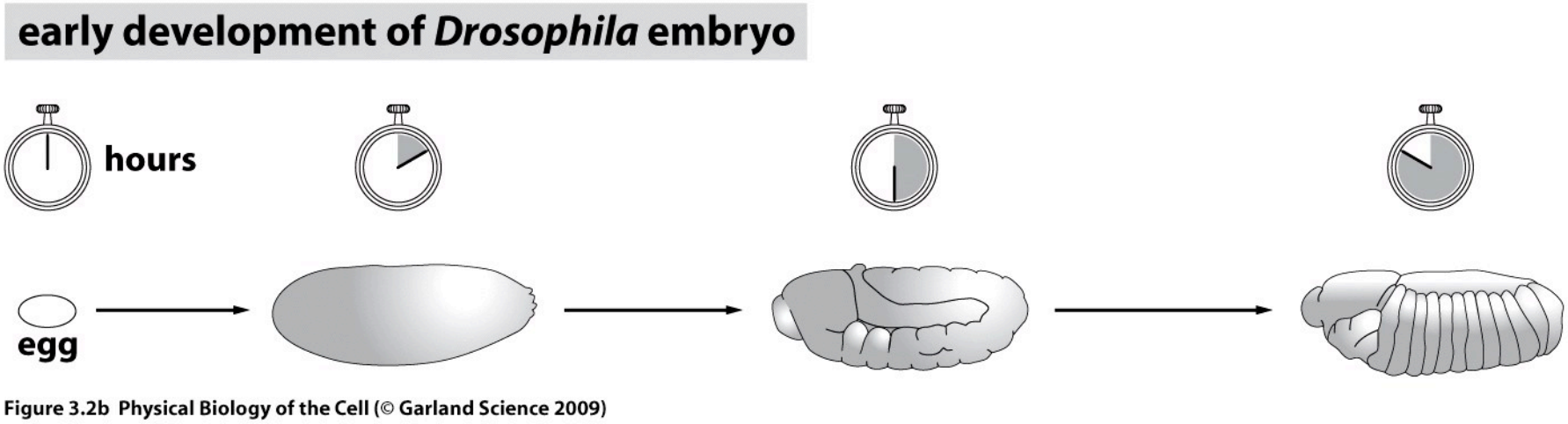
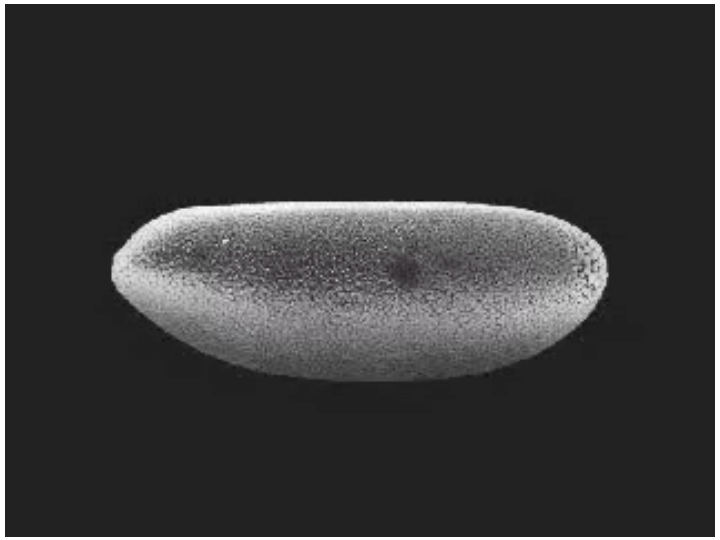


Figure 3.2b Physical Biology of the Cell (© Garland Science 2009)

- *Development of the fly embryo: ~10 hrs*
- *a single cell → 1000s of cells with particular spatial positions and functions*
- *a dramatic part of embryonic development: the process of gastrulation (a series of folding events in the embryo → formation of the future gut)*



TIME SCALES IN EMBRYONIC DEVELOPMENT

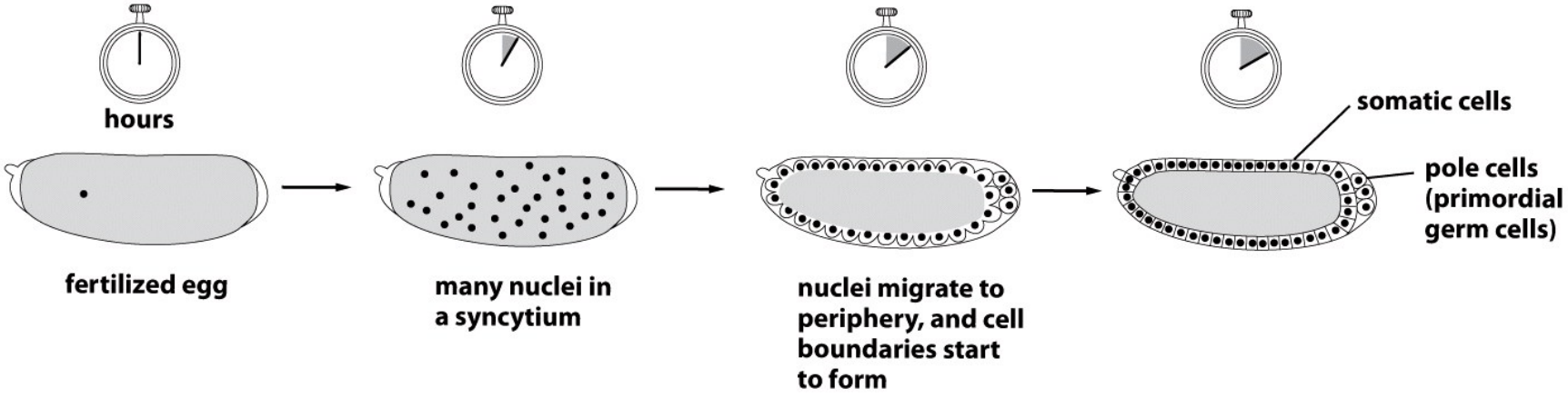


Figure 3.22 Physical Biology of the Cell (© Garland Science 2009)

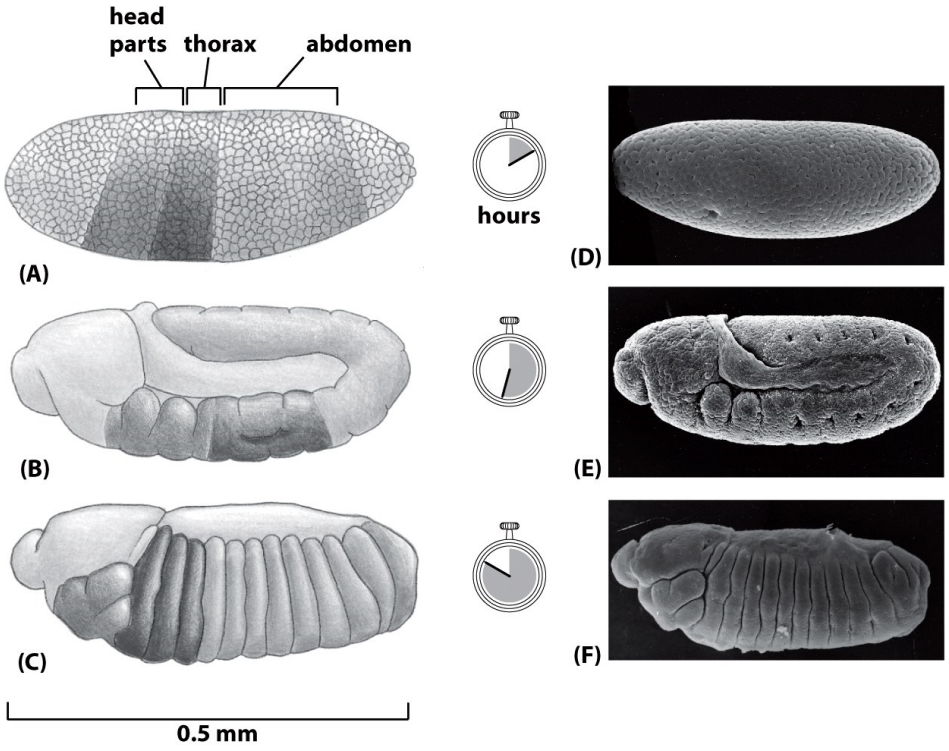
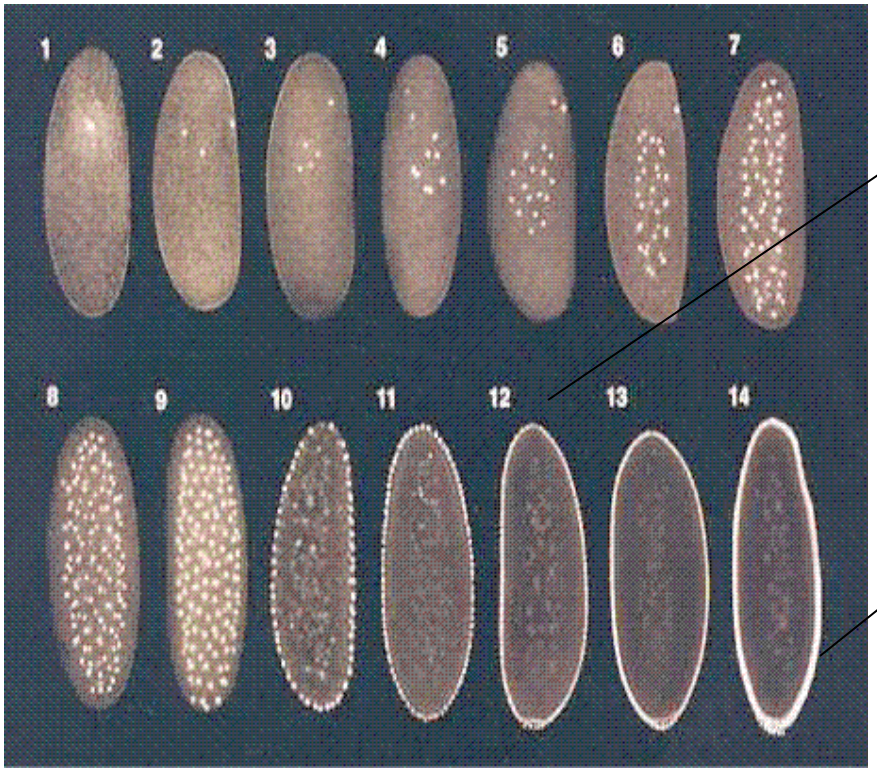
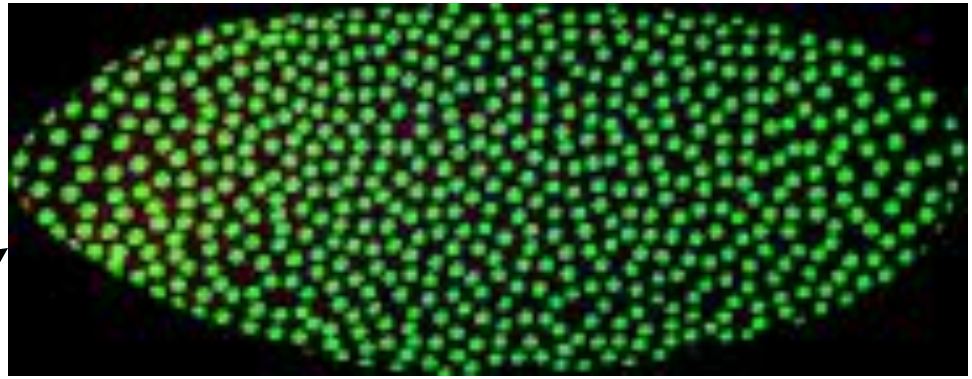


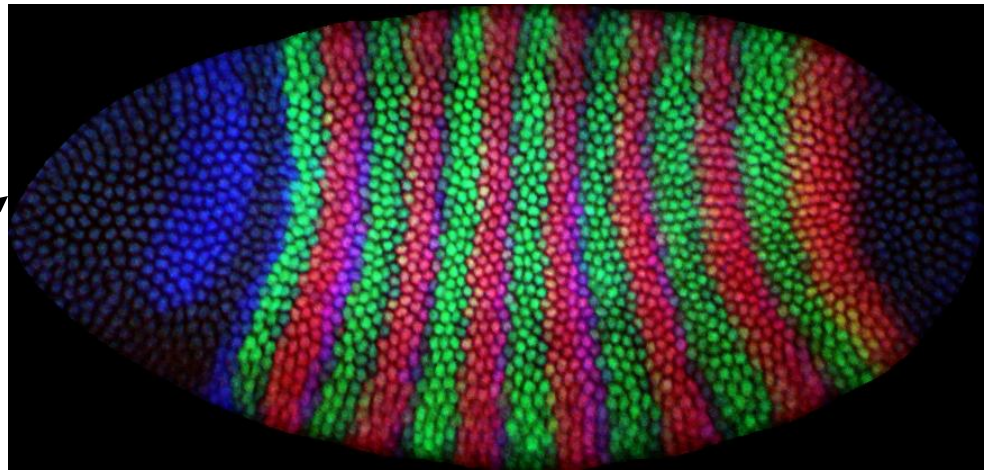
Figure 3.23 Physical Biology of the Cell (© Garland Science 2009)

PATTERNS OF GENE EXPRESSION IN THE FLY EMBRYO

Development in a box!



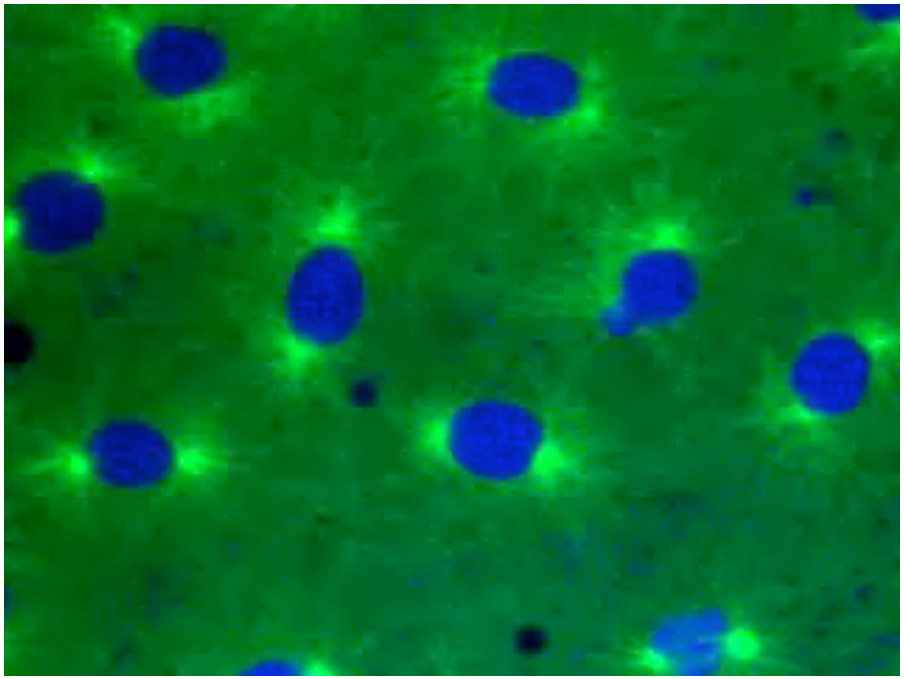




Doubling time = 8min

How are these spatial patterns of gene expression established?

CELL DIVISION IN THE FLY EMBRYO



BIOLOGICAL TIME SCALES IN POWERS OF 10: 10s OF SECONDS

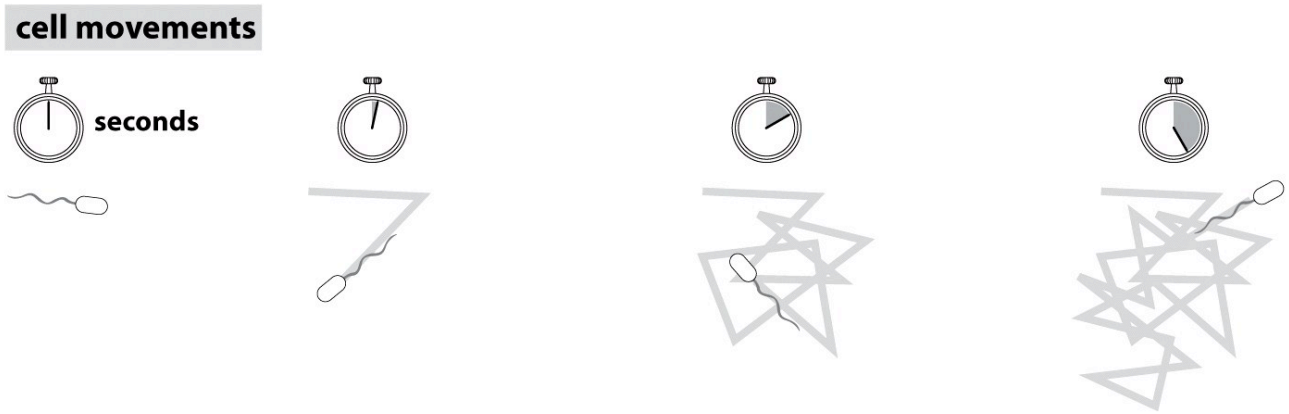
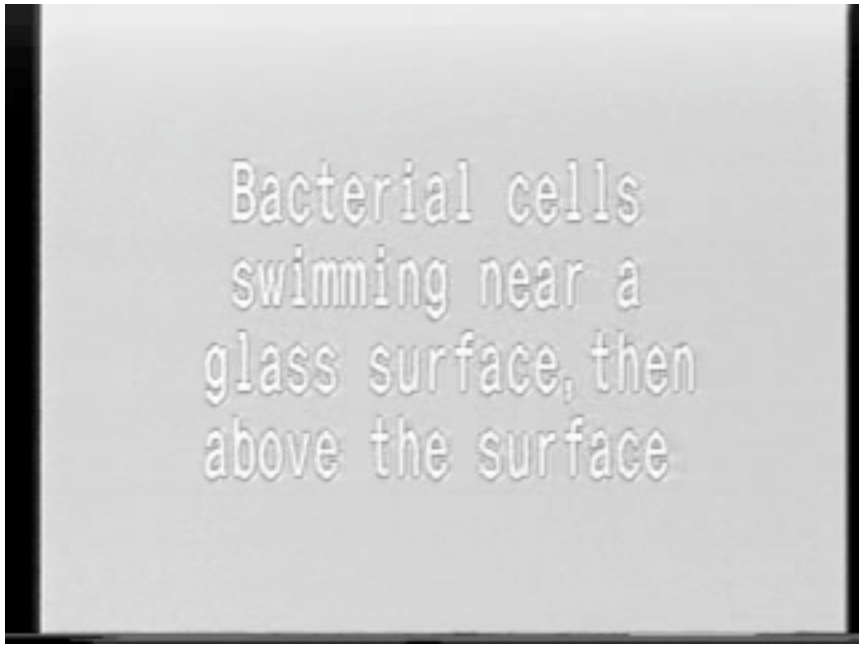


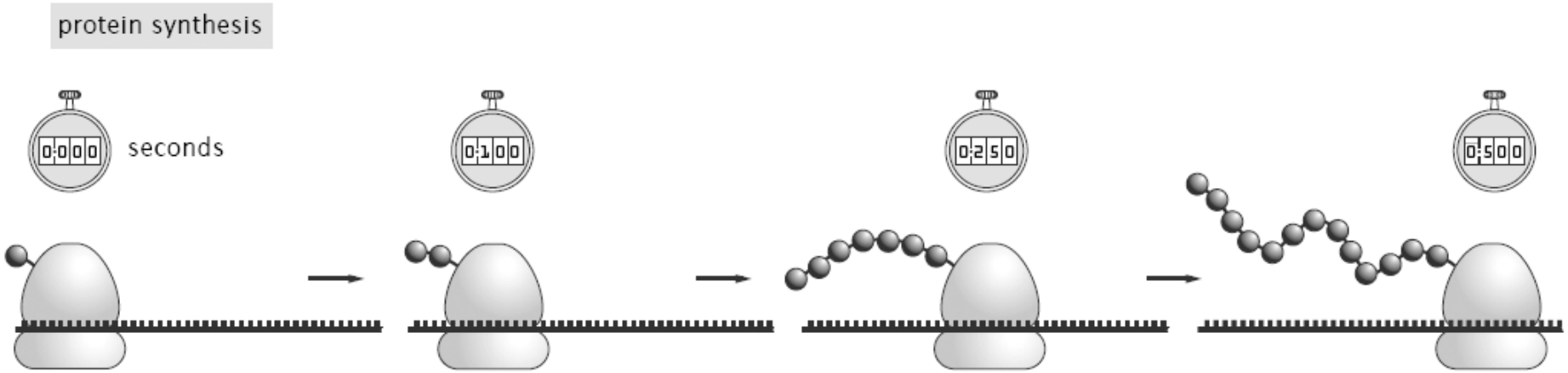
Figure 3.2d Physical Biology of the Cell (© Garland Science 2009)

(courtesy of Howard Berg)



- *a swimming E.coli: episodes of directed motion, punctuated by rapid directional changes*

BIOLOGICAL TIME SCALES IN POWERS OF 10: DECISECONDS



- *AAs incorporation during protein synthesis*
- *process which any cell must undertake to make a new cell*

BIOLOGICAL TIME SCALES IN POWERS OF 10: DECISECONDS

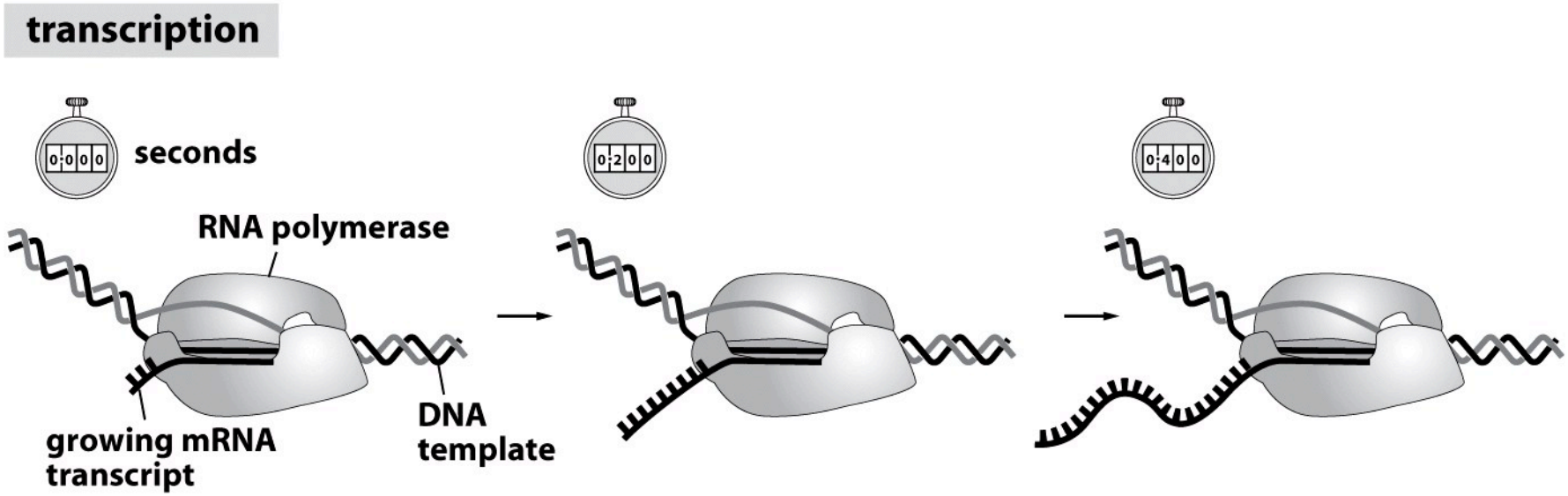
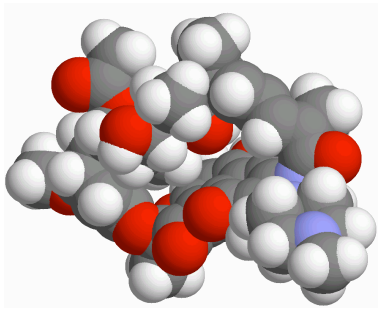


Figure 3.2f Physical Biology of the Cell (© Garland Science 2009)

- *synthesis of mRNA molecules as faithful copies of the nucleotide sequence in the DNA*
- *polymerization process catalyzed by enzyme RNA polymerase*
- *the incorporation by RNA polymerase of nucleotides onto the mRNA during transcription happens a few times faster than AA incorporation by ribosomes during protein synthesis*

How fast is the information read out?

- ***Amazing microscopy images of transcription "Christmas trees" at various times after treatment with an antibiotic.***
- ***Rifampin stops transcription initiation, but not elongation.***
- ***This was used to estimate the rate at which RNA polymerase produces the messenger RNA molecule.***



(L. S. Gotta et al, J. Bacteriol., 1991)

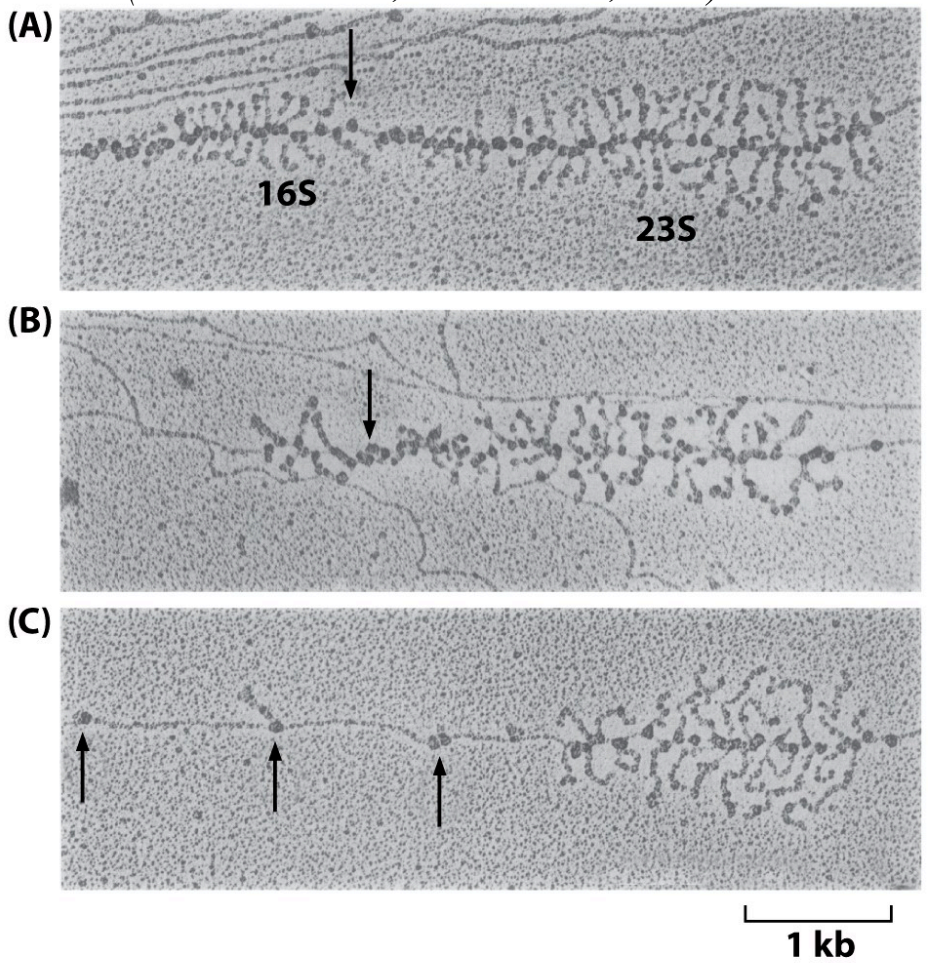


Figure 3.29 Physical Biology of the Cell (© Garland Science 2009)

BIOLOGICAL TIME SCALES IN POWERS OF 10: MILLISECONDS

- *many proteins are able to operate at time scales much faster than the machinery carrying out the central dogma operations*

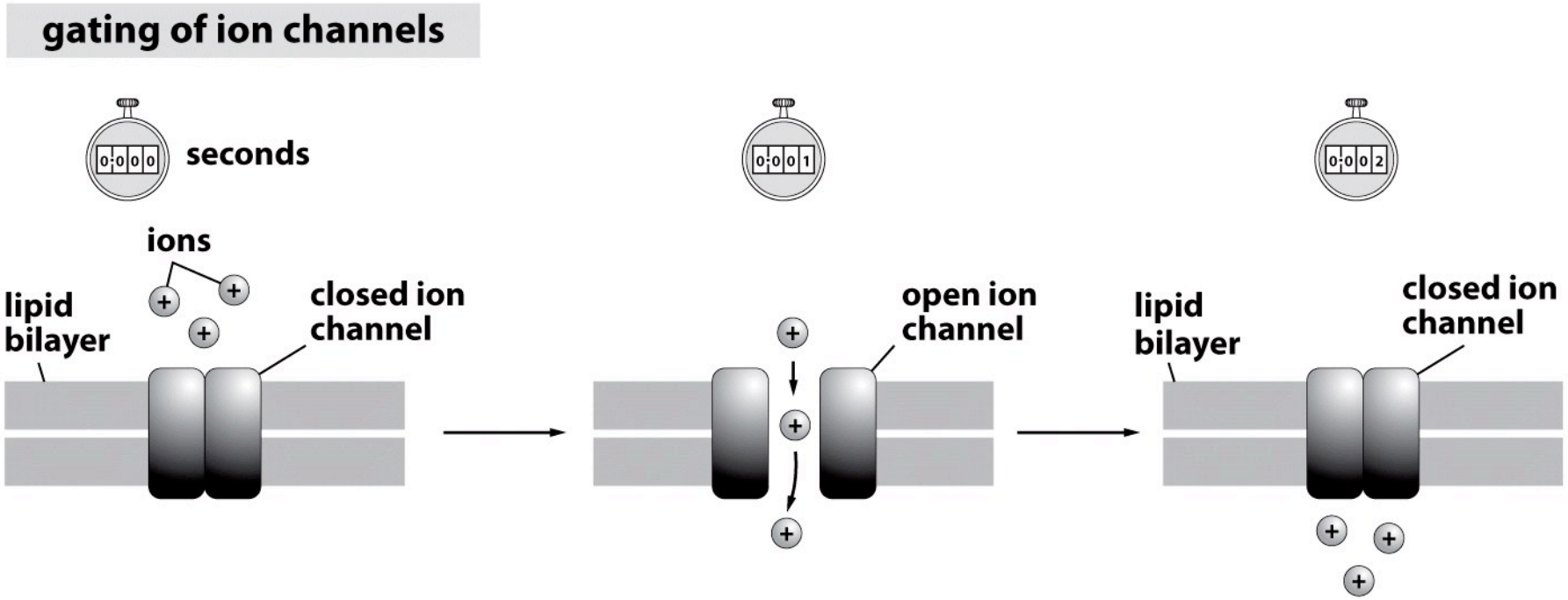


Figure 3.2g Physical Biology of the Cell (© Garland Science 2009)

- *E.g., many bio processes are dictated by the passage of ions across ion channels (scale of msec)*

BIOLOGICAL TIME SCALES IN POWERS OF 10: μSECONDS

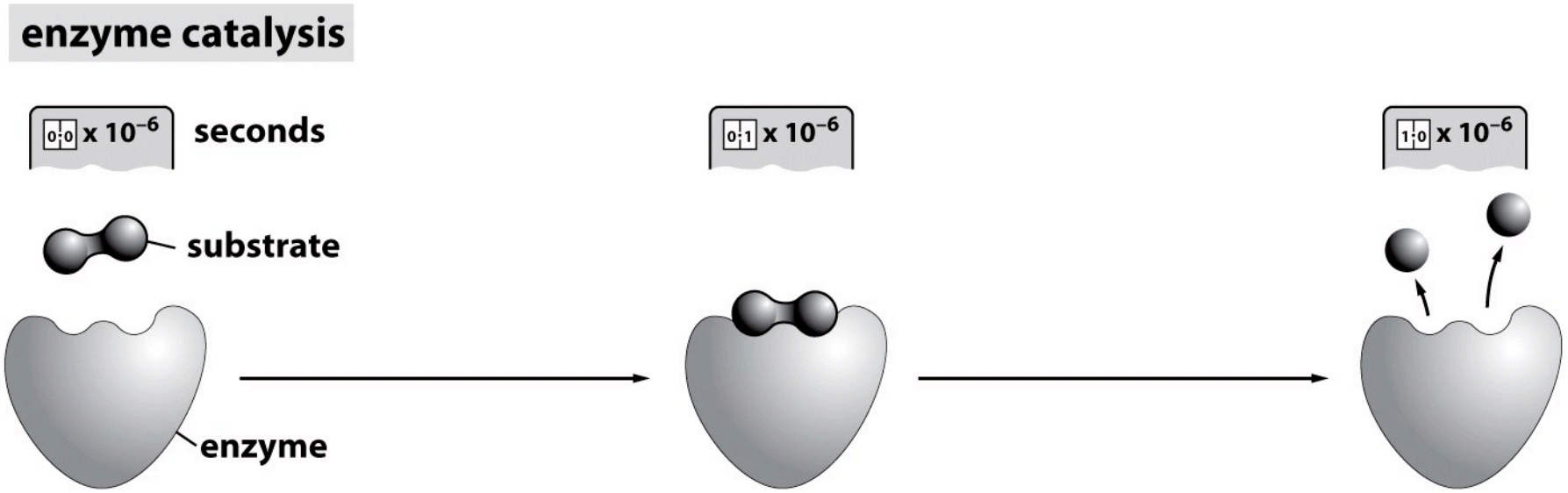
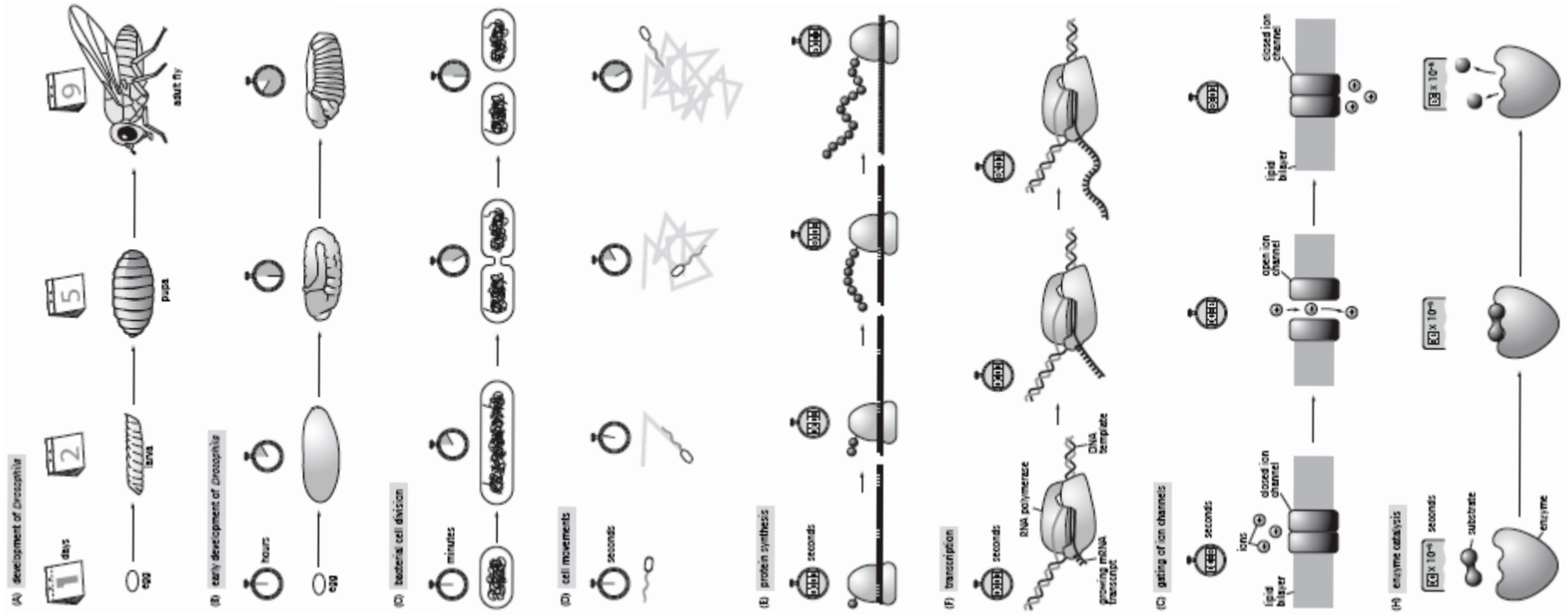


Figure 3.2h Physical Biology of the Cell (© Garland Science 2009)

- *Enzyme kinetics (μsec and faster)*
- *Note: these time scales represent a general rule of thumb; turnover rates for individual enzymes vary: 0.5 s^{-1} to 600,000 s^{-1}*

HIERARCHY OF BIOLOGICAL TIME SCALES: SUMMARY



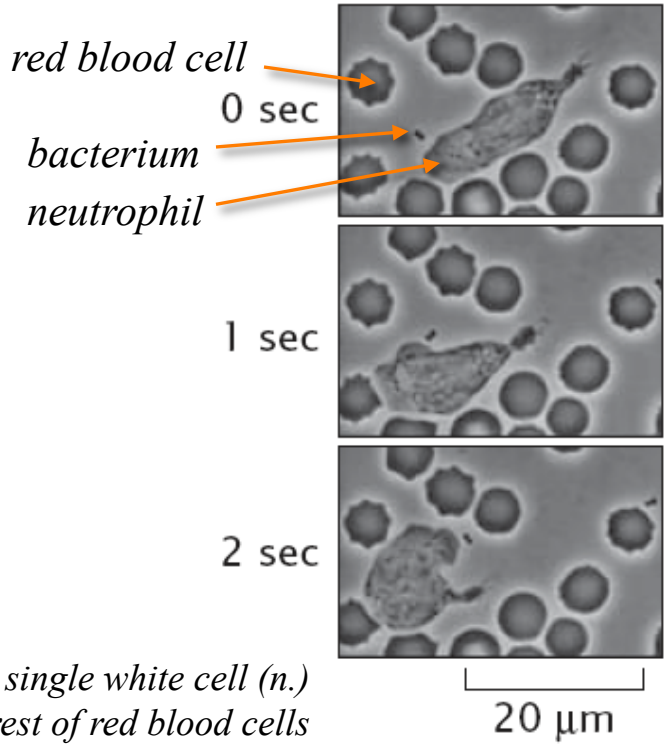
HOW DO WE KNOW WHAT WE KNOW?

MEASUREMENTS OF BIO TIME

- Exp. on the dynamics of cells & molecules populating them: tracking transformations.

Strategy 1	Typ'l time scale	Types of processes	Example
Direct observation	msec-hrs	Individual transformations	Cell crawling

- *observe the process unfold, record the absolute t at which transformation occurs*
- *easy to do for time scales of min to hrs, spatial scales that can be observed with a light microscope or the unaided human eye*
- *difficult to measure t for events that are very fast, very slow, very small, very large*
- *past decade: vast exp.improvements in direct or near-direct observations of single molecules*



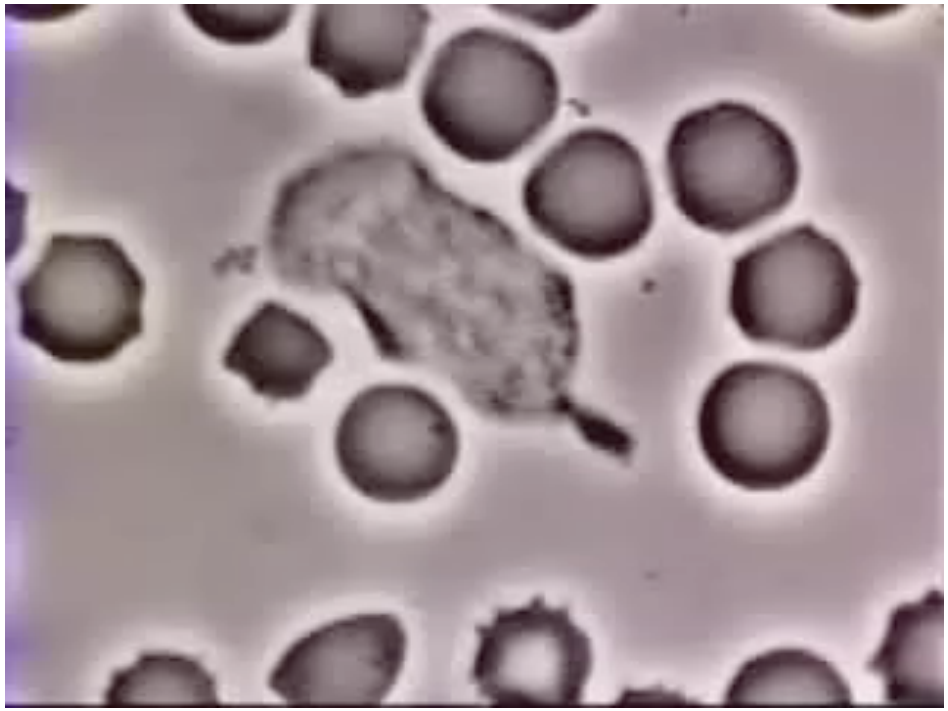
3 frames from a video sequence taken by D.Rogers in 1950s of a single white cell (n.) pursuing a bacterium through a forest of red blood cells

How do we know what we know?

Measurements of Bio Time

Strategy 1

Direct observation



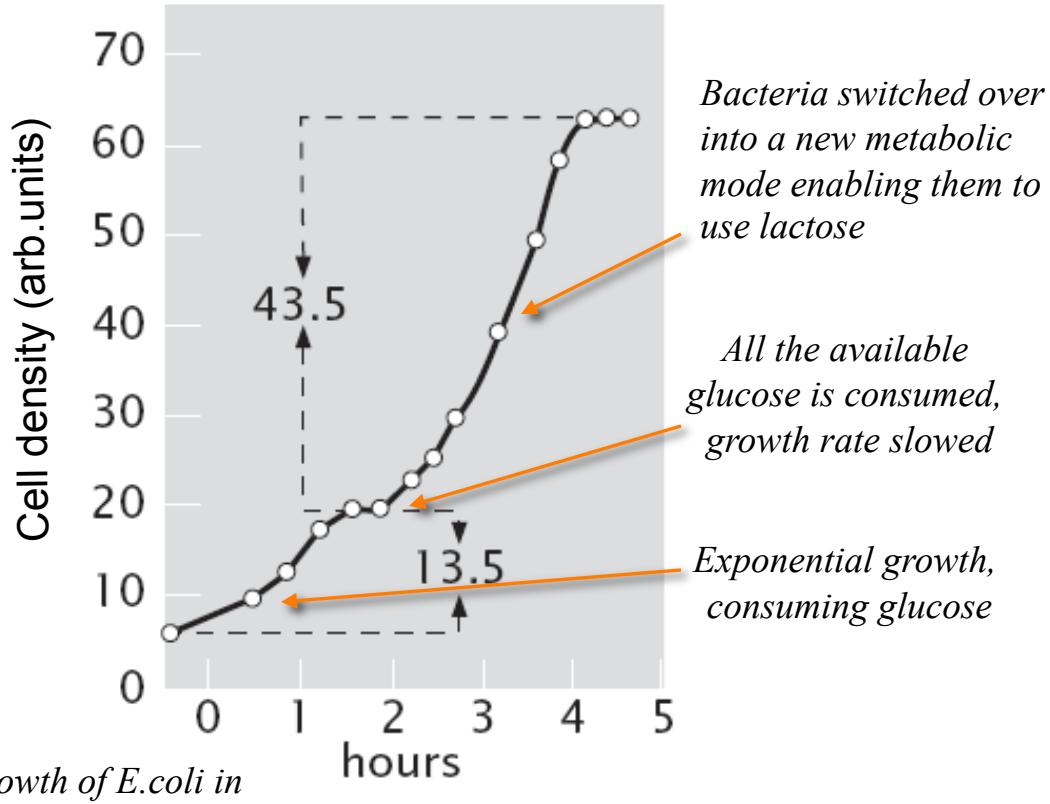
Direct observation: a single white cell (n.) pursuing a bacterium through a forest of red blood cells

HOW DO WE KNOW WHAT WE KNOW?

MEASUREMENTS OF BIO TIME

Strategy 2	Typ'l time scale	Types of processes	Example
Fixed time points	μsec-yrs	Population changes	Bacterial growth curve

- *draw individuals from a population at given time intervals, examine their properties at these fixed t points*
- *E.g., bacterial growth (hrs to days)*
- *E.g., embryonic development for model organisms (days to weeks)*
- *Small spatial scale & fast time, method of stopped-flow kinetics: mix enzyme and substrate → squirt the mixture into a denaturing acid bath after fixed Δt => follow enzymatic events*



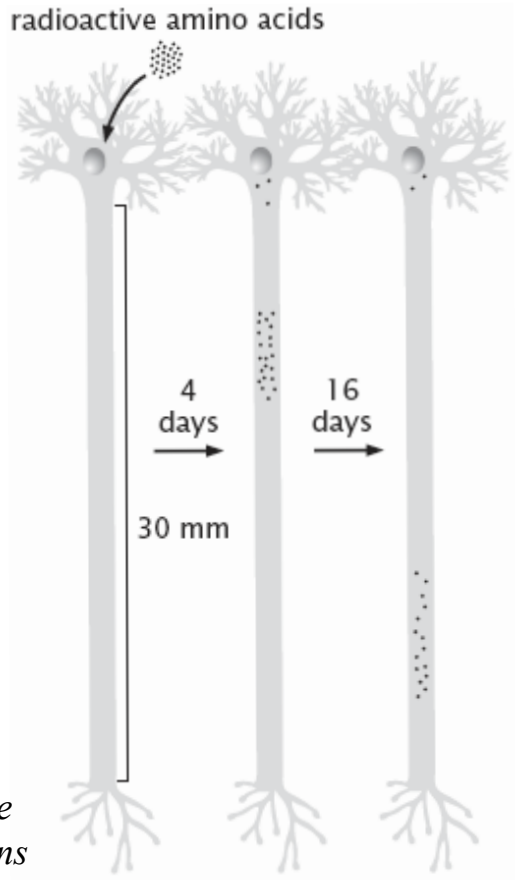
The experiment (by Monod) is a classic, tracked the growth of E.coli in a single culture when two diff. nutrient sugars were mixed together

How do we know what we know?

Measurements of bio time

Strategy 3	Typ'l time scale	Types of processes	Example
Pulse-chase	min-days	continuous (*e.g., metabolism, transport*)	Axonal transport

- *Many bio processes operate in a continuous fashion*
- *E.g., bacteria constantly take in sugar. Glycolysis: 1 molecule of glucose → 2 molecules of pyruvate*
- *=> difficult to measure how long a conversion process takes*
- *Pulse-chase: briefly feed glucose tagged with radioactive C, feed with nonradioactive glucose => examine cells at various Δt to see whether glucose or pyruvate contain radioactive C => t of conversion*
- *The same strategy: transport in neurons*

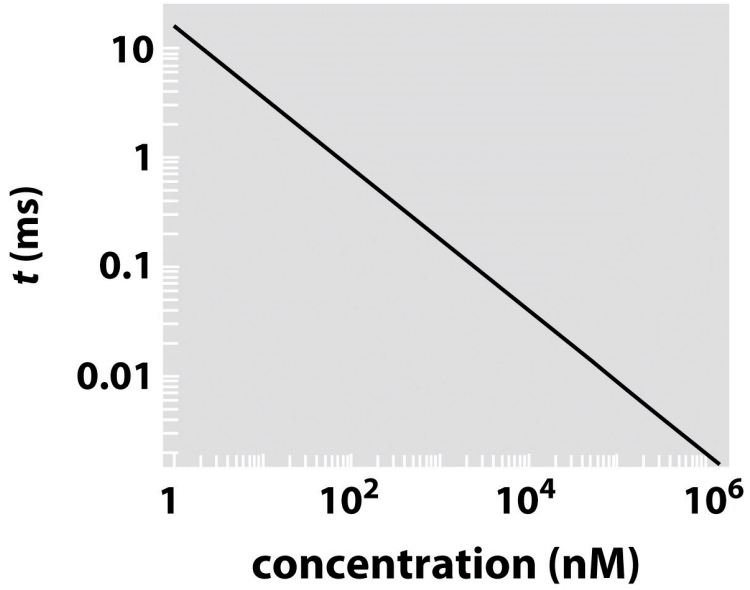


Label proteins at their point of synthesis in a neuron cell body with a pulse of radioactive AAs => a chase of unlabeled AAs => measure the rate of continuous transport in neurons

BEATING THE DIFFUSION SPEED LIMIT

- *=> impossibly long time scales associated with diffusion over large distances*
- *Nature's solution: active transport*

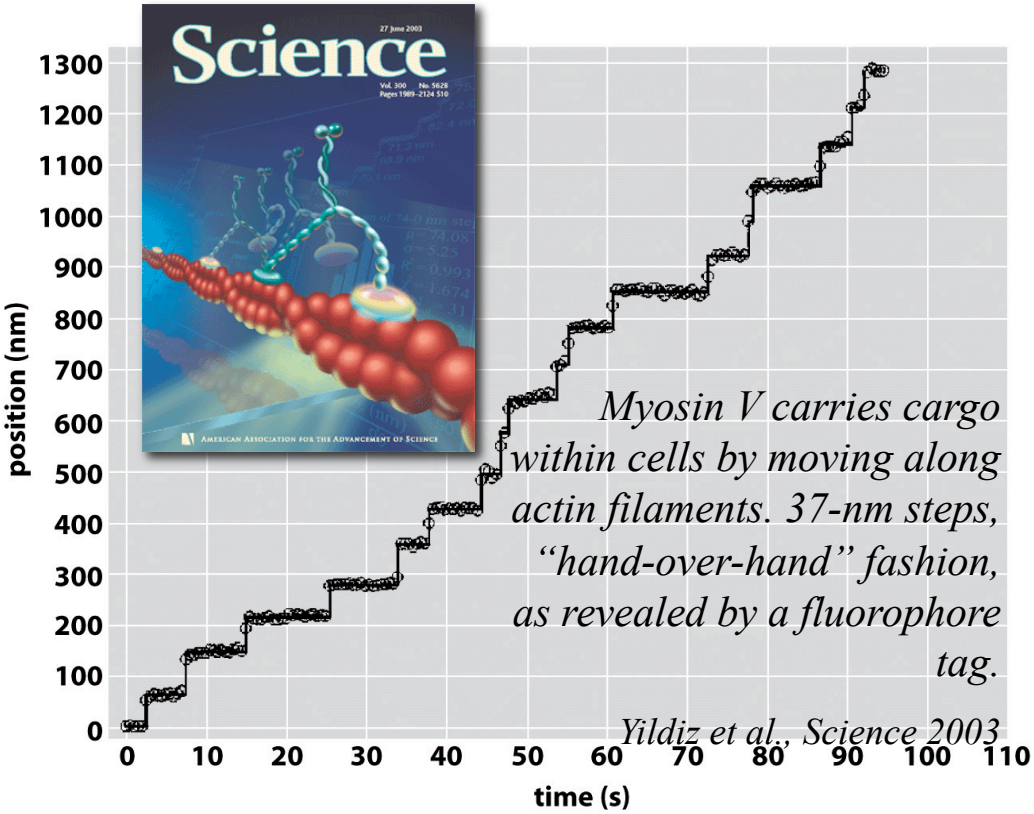
Mechanism #1: passive diffusion



c measured in diffusion times. The characteristic for diffusion-mediated molecular encounters depends upon the c of the diffusing species

Mechanism #2: active transport

ATP consumed => motor molecules carry out directed motion



Myosin V carries cargo within cells by moving along actin filaments. 37-nm steps, "hand-over-hand" fashion, as revealed by a fluorophore tag.

Yildiz et al., Science 2003

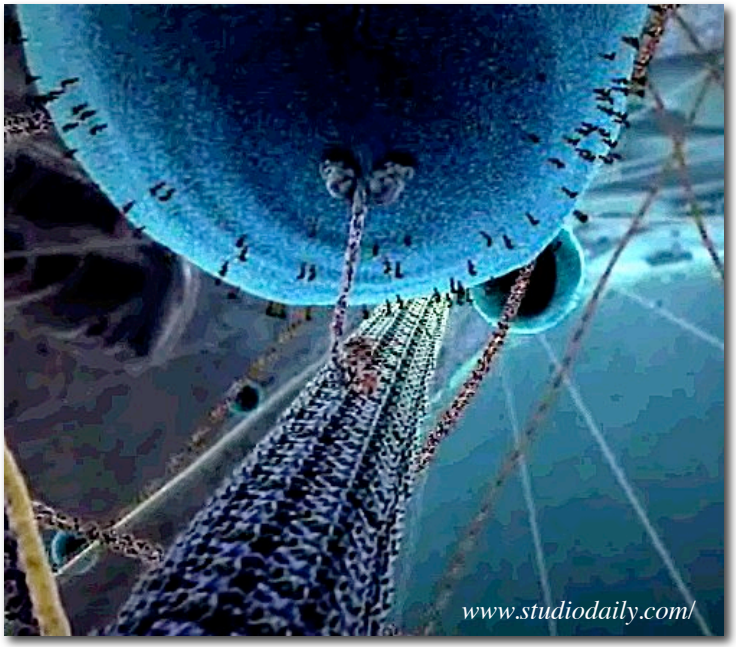
MOVING PROTEINS FROM HERE TO THERE (PART 2)

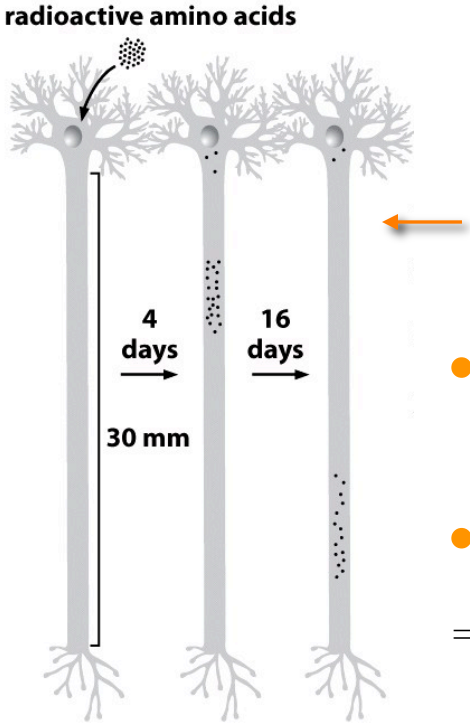
Estimate the transport time for kinesin motor moving on a microtubule over the distance of 10 cm:

- *speed of kinesin in a living cell: 1 μm/s*

=> $t_{axon} \approx 10^5$ μm / (1 μm/s) = 10^5 s ≈1 day

comp. with passive diffusion: 3 yrs





Classic experiment traces the time evolution of radioactively labeled proteins in a neuron

- *Fig.: radio-labeled proteins travel ≈18 mm in 12 days*

=> <v> ≈ 20 nm/s

- *Observed axonal transport speed for single motors: 200 nm/s or more*

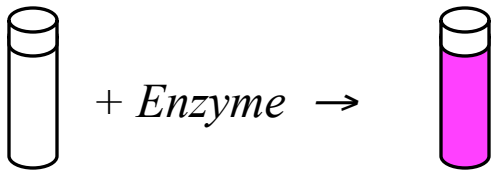
=> Motors are not perfectly "processive" (fall off) => <v>↓

How do we know what we know?

Measurements of bio time

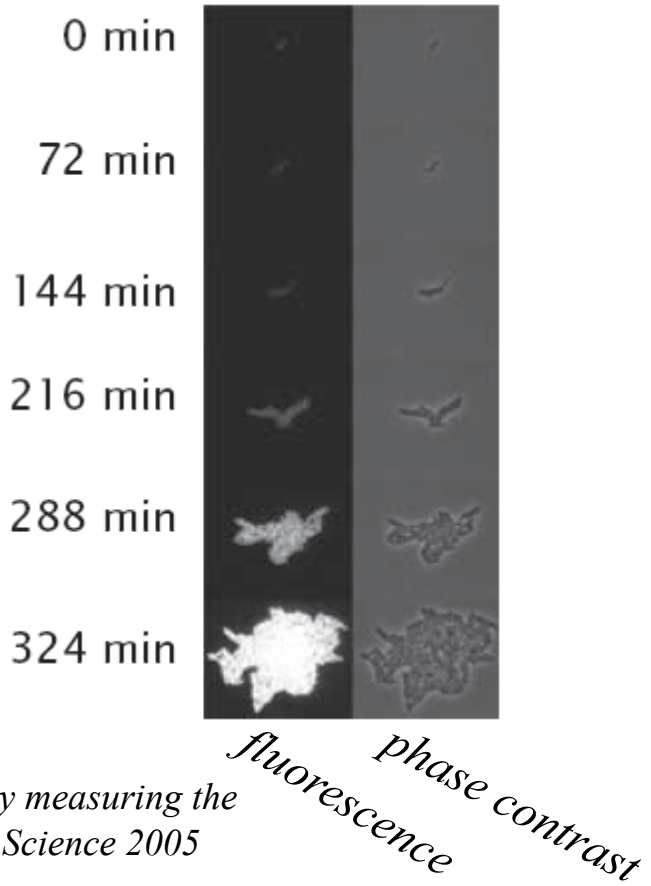
Strategy 4	Typ'l time scale	Types of processes	Example
Product accumulation	min-days	Biosynthetic or enzymatic	GFP expression

Idea:



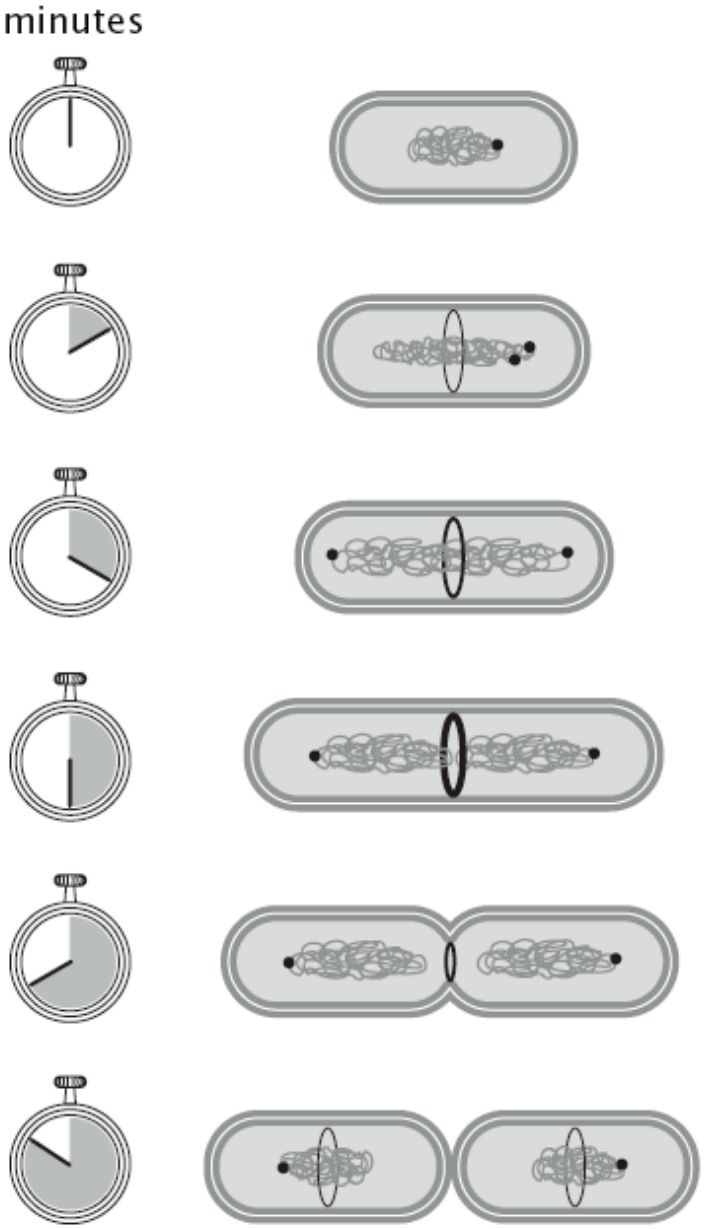
colorless substrate *colored product*

- *can be used for performing rate measurements in living cells*
- *E.g., expressing GFP downstream of a promoter of interest*
- *When the promoter is induced (<=> the gene of interest is turned on) GFP begins to accumulate => amount of fluorescence can be converted into N of GFP molecules => reporter for promoter activity*



The rate of gene transcription in a bacterial cell is inferred by measuring the amount of GFP present as a function of t. Rosenfeld et al., Science 2005

AN IDEALIZED BACTERIAL CELL CYCLE



$$A_{cross\text{-}section} \approx const,$$

$$l_f \approx 2l_i$$

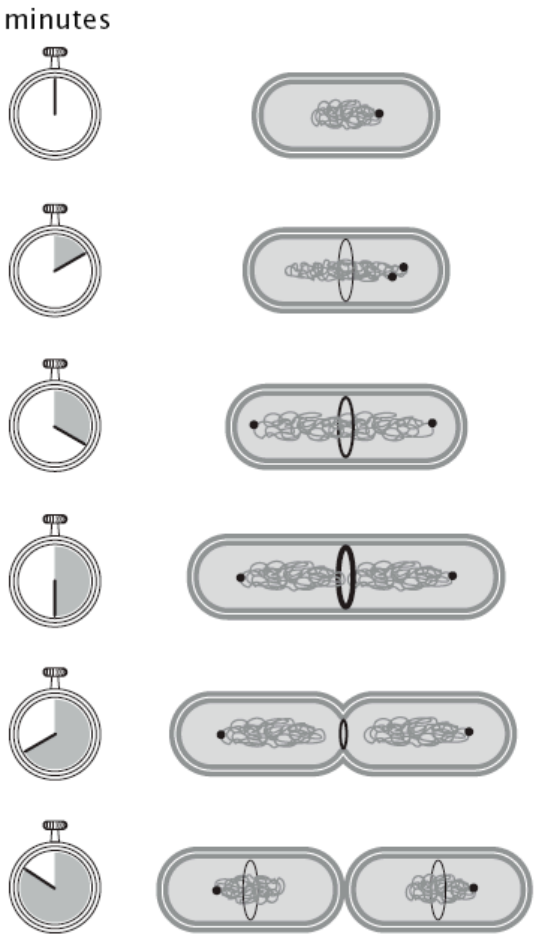
$$\Rightarrow V_f \approx 2V_i$$

$$\tau_{Binary\ fission} \sim 1\ hr$$

- *a newborn cell has a single chromosome with a single origin of replication*
- *duplication of the origin, DNA replication around the circular chromosome, proteins FtsZ form a ring (future septum site)*
- *DNA replication proceeds, the cell elongates, two origins take up residence at the opposite poles*
- *the septum begins to close down*
- *the two chromosomal masses are physically separated into two daughter cells…*
- *… where the cycle can begin anew*

THE E.COLI CELL CYCLE AS THE STANDARD STOPWATCH

- *E.coli*: standard measuring stick, standard stopwatch
- Standards for bacterial cell cycle:
  - minimal medium (glucose as the sole C source)
  - 37°C
  - 3000 s (=50 min)



THE MACHINES OF THE CENTRAL DOGMA

- Prime example of procedural *t*: the processes of the central dogma.
- DNA → RNA → proteins

- *Replication: copying genome (DNA → DNA)*

must take place before a cell divides;

mediated by the replisome (= enzyme DNAP [incorporates new nucleotides onto a nascent DNA] + helicases [unwind DNA] + primases [prime the polymer-n reaction])

- *Transcription: synth.of mRNA (DNA → RNA)*

template for protein synthesis;

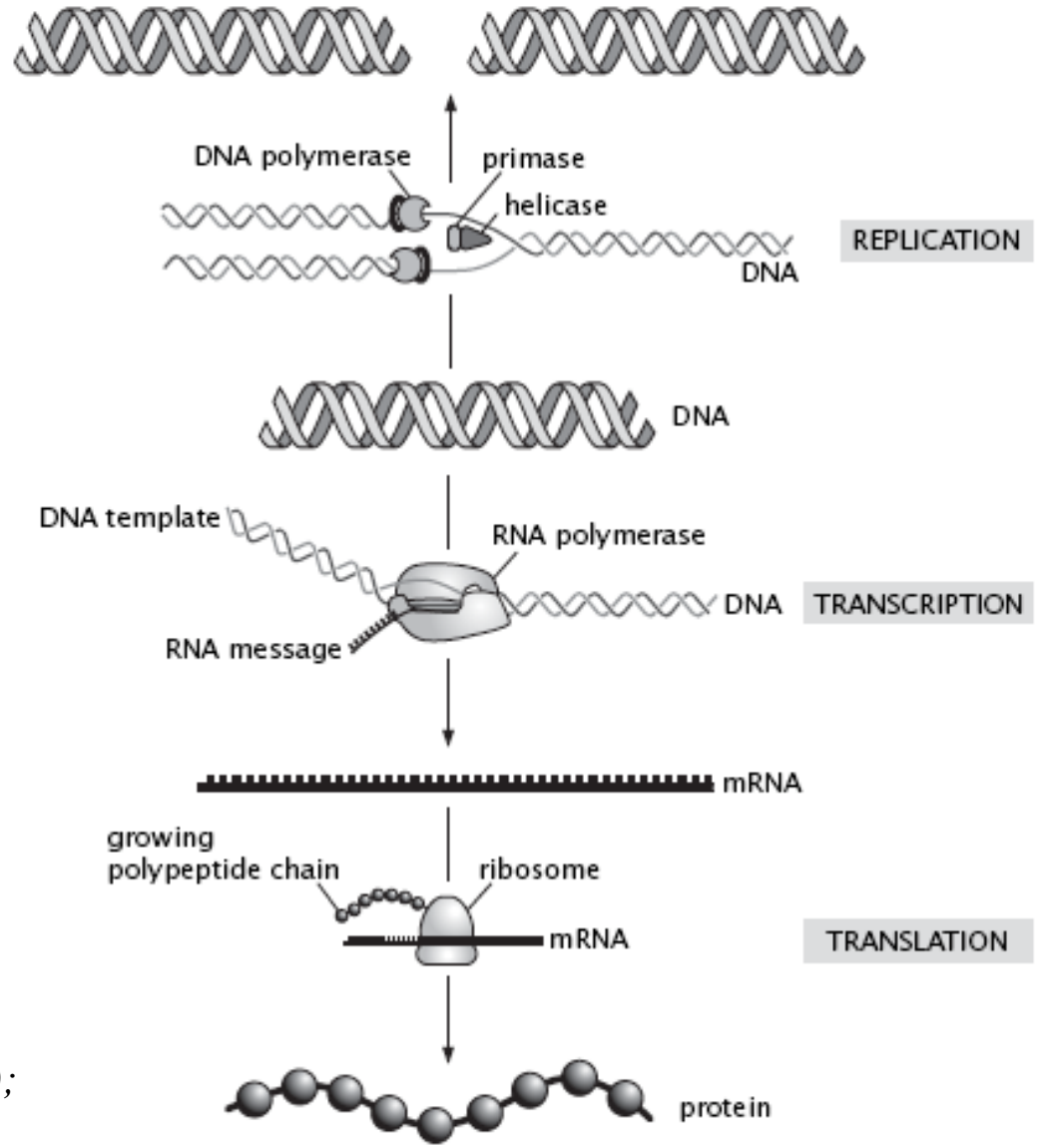
carried out by RNAP

euk.: occurs in the nucleus => mRNA export

- *Translation: (RNA → protein)*

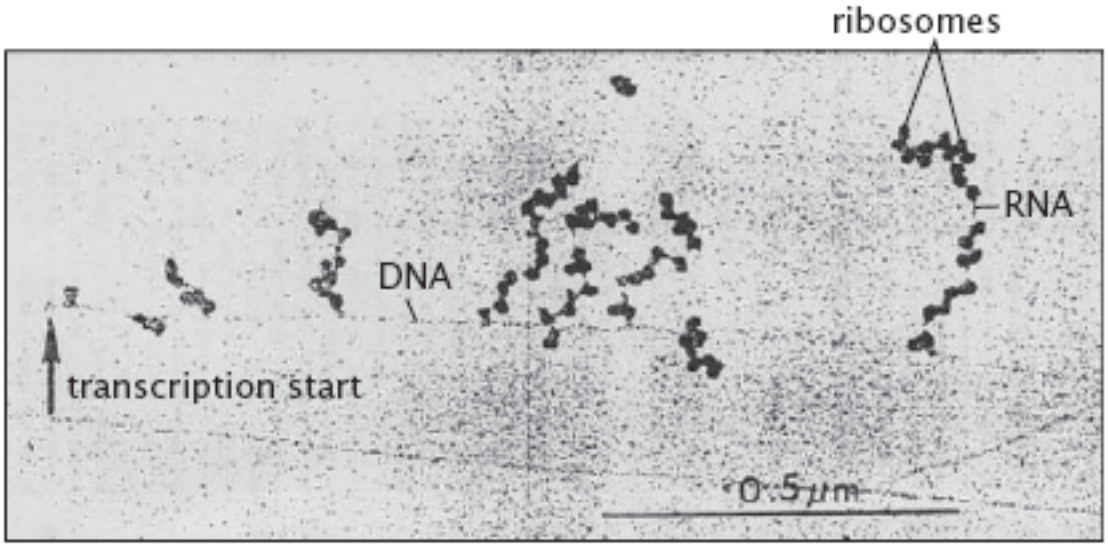
Mediated by the ribosome (RNA+protein comp.);

Transfer of AAs from tRNA onto polypept. chain



THE MACHINES OF THE CENTRAL DOGMA

- Nascent mRNA molecules in bacteria are immediately engaged by ribosomes: protein translation occurs before transcription is even finished.



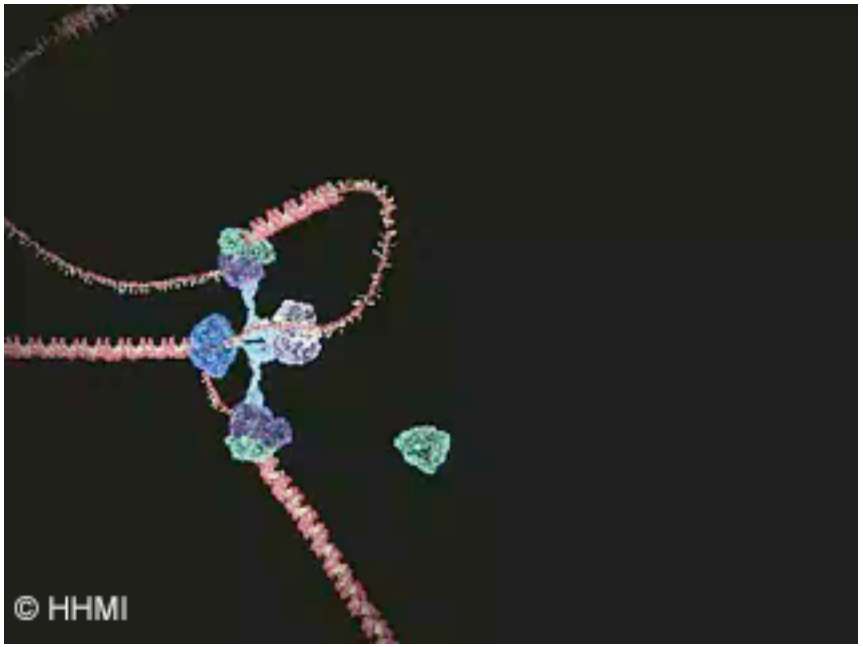
Electron microscopy image of simultaneous transcription and translation. (Miller et al., Science 1970)

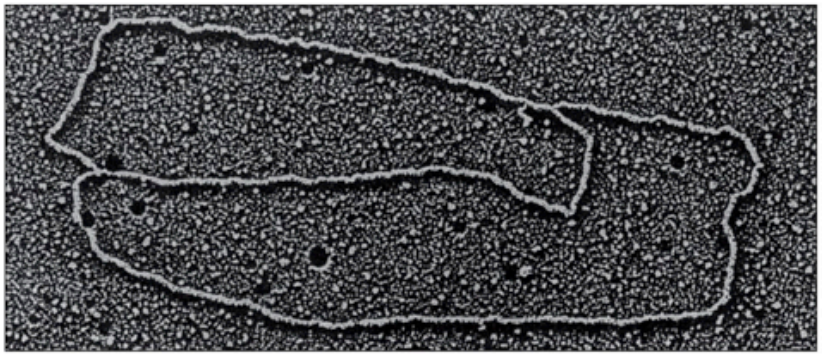
- Timing of the replication, transcription and translation is dictated by the intrinsic rate at which the machines carry their polymer-n reactions.
- Each of the processes: N "identical" reactions, each takes Δt on average to perform

TIMING THE MACHINES OF THE CENTRAL DOGMA

- DNA replication in bacteria is undertaken by 2 replication complexes which travel in opposite directions away from the origin of replication on the circular chromosome.

Estimate the rate of the DNA polymerase complex:





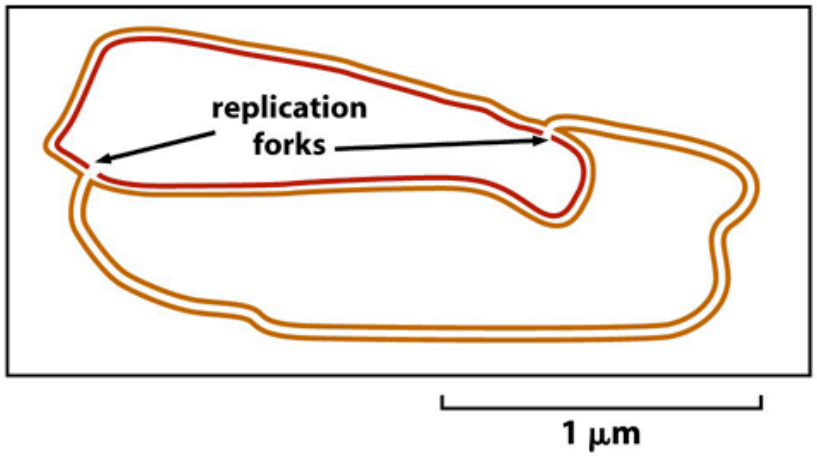


Figure 5-6 Molecular Biology of the Cell 5/e (© Garland Science 2008)

- *E.coli* genome ≈ 5×10^6 bp, $\tau_{cell} \approx 3000$s

rate of DNA synthesis: 2000 bp/s

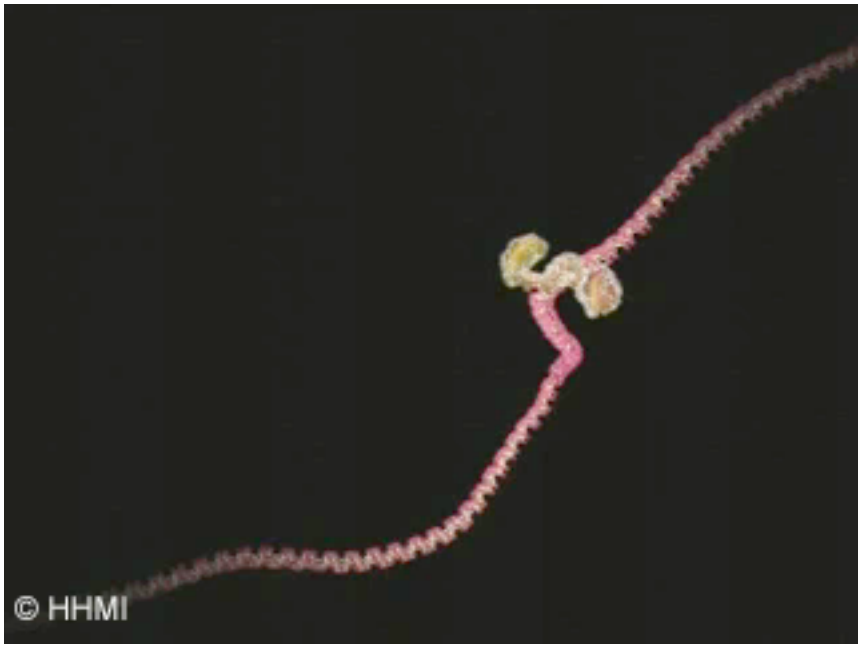
<=> 1000 bp/s per replisome

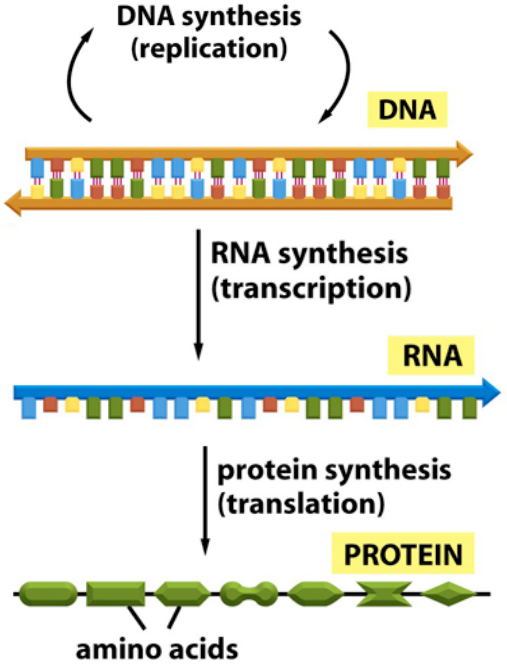
(biochem.experiment: 250-1000 bp/s)

Parental strands: orange, newly synthesized strands: red

TIMING THE MACHINES OF THE CENTRAL DOGMA

Estimate the time to make a transcript (mRNA) in bacteria.



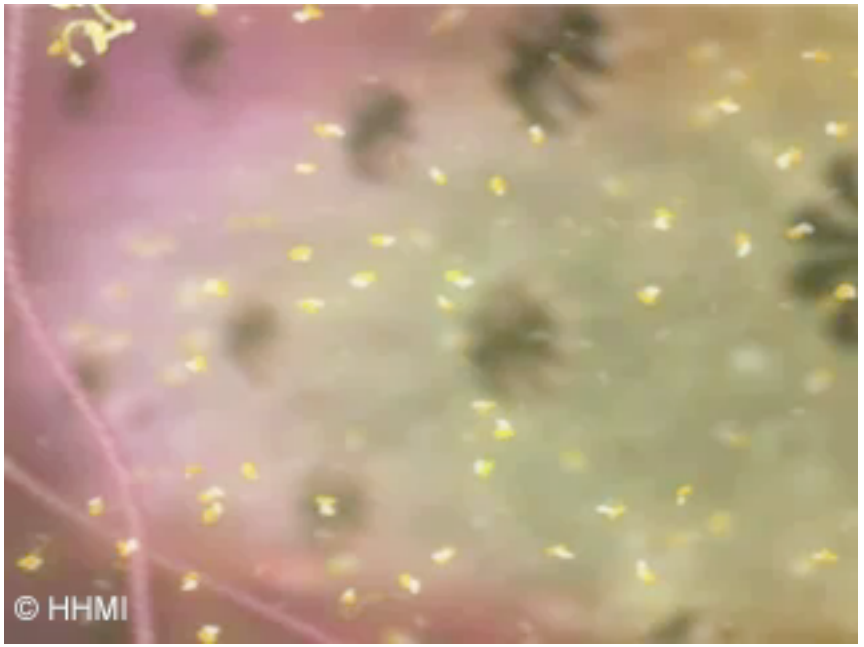


- Typical protein: 300 AAs, 3 bases needed to specify each AA => l_{mRNA}~1000 bp
- Characteristic transcription rate (exp.): ~ 40 nucleotides/s

=> $t_{mRNA} \approx 25$ s

TIMING THE MACHINES OF THE CENTRAL DOGMA

Estimate the mean time to synthesize a typical protein.



Translation in Eukaryotes

- *E.coli*: $N_{protein} \approx 3 \times 10^6$, each protein: 300 AA,

$\tau_{cell} \approx 3000$ s

=> Rate of AA incorporation by the ribosome:

$dN_{AA}/dt \approx (9 \times 10^8 \text{ AA}) / 3000 \text{ s} \approx 3 \times 10^5 \text{ AA/s}$

- $N_{ribosome} \approx 20{,}000$ => rate per ribosome: 15 AA/s (exp.: 25 AA/s)

=> $t_{protein} \approx 20$ s

- Rate of protein synth. by the ribosome < rate of mRNA synthesis by RNA polymerase.
- Simultaneous translation of a single mRNA by multiple ribosomes
- => When considering the net rates of processes in cells: *N* of mol. players + the intrinsic rate.

TIMING E.COLI

Estimate the rates of various processes in the *E.coli* cell cycle

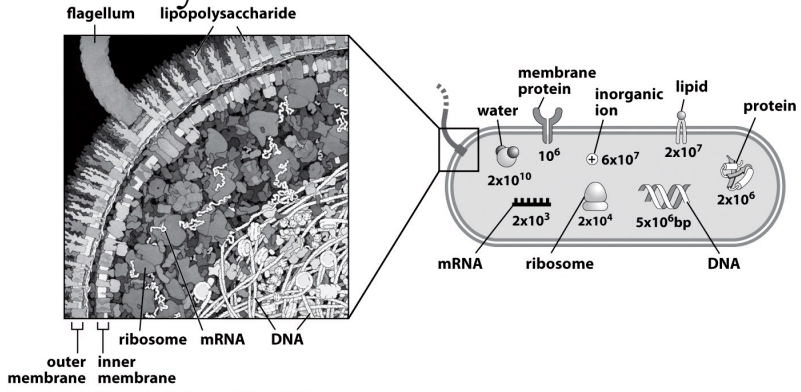


Figure 2.2 Physical Biology of the Cell (© Garland Science 2009)

- *E.coli* genome: 5×10^6 bp
- time of the cell cycle: $\tau_{cell} \approx 3000$ s

Rate of replication:

$$dN_{bp}/dt \approx N_{bp}/\tau_{cell} \approx 5 \times 10^6 \text{ bp}/ 3000 \text{ s} \approx 2000 \text{ bp/s}$$

Rate of protein synthesis:

$$dN_{protein}/dt \approx N_{protein}/\tau_{cell} \approx 3 \times 10^6 \text{ protein}/ 3000 \text{ s} \approx 1000 \text{ proteins/s}$$

Rate of lipid synthesis:

$$dN_{lipid}/dt \approx N_{lipid}/\tau_{cell} \approx 2 \times 10^7 \text{ lipids}/ 3000 \text{ s} \approx 10{,}000 \text{ lipids/s}$$

TIMING E.COLI (CONT'D)

- Control of the water content within the cell - ?

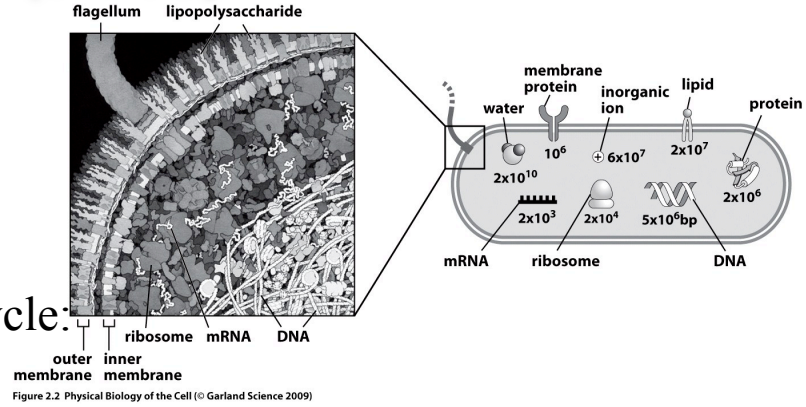


Figure 2.2 Physical Biology of the Cell (© Garland Science 2009)

Estimate the rate of water uptake during the cell cycle:

$$dN_{H2O}/dt \approx N_{H2O}/\tau_{cell} \approx 2 \times 10^{10} \text{ waters}/ 3000 \text{ s} \approx 7 \times 10^6 \text{ waters}/ \text{s}$$

Estimate the average mass flux across the cell membrane:

Flux = amount of m crossing unit A per unit t <=>

$$j_{water} \approx (dN_{H2O}/dt) / A_{E.coli} \approx (7 \times 10^6 \text{ waters/s}) / (6 \times 10^6 \text{ nm}^2) \approx 1 \text{ water}/(\text{nm}^2\text{s})$$

Note: This mass transport is mediated primarily by proteins which are distributed throughout the membrane.

THIRD TOPIC
ENERGY AND FORCE SCALES IN BIOLOGY

http://media.bigoo.ws/content/image/funny/funny_407.jpg

ATP AS THE ENERGY CURRENCY

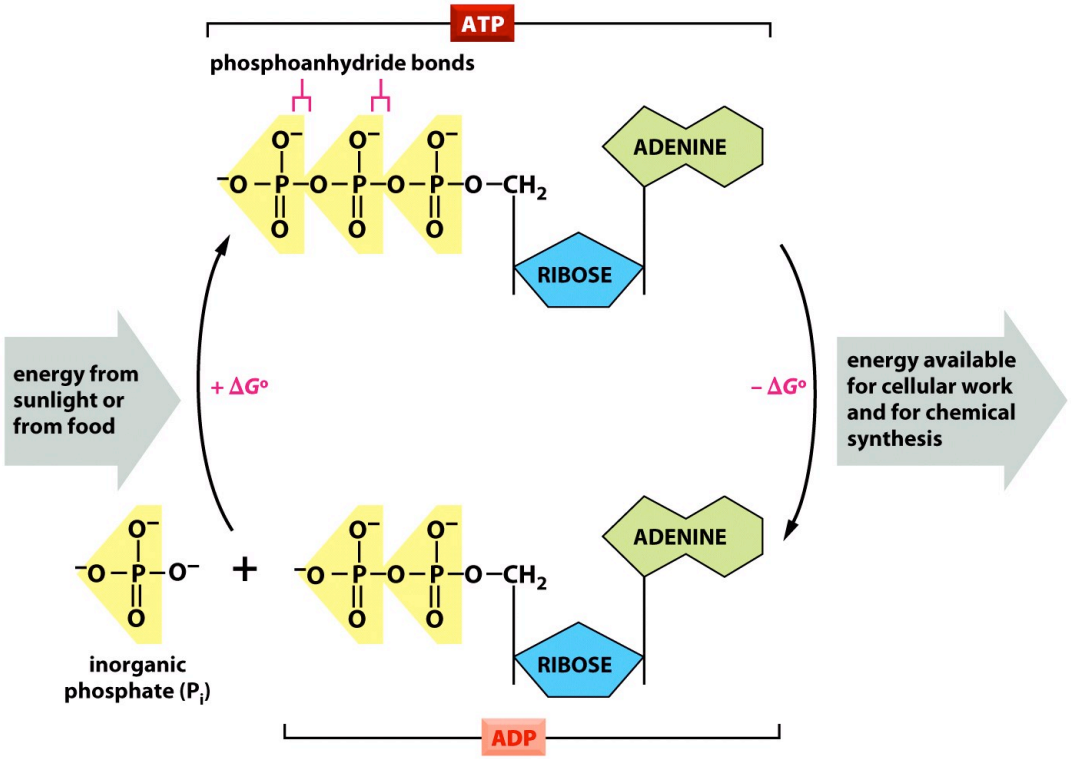
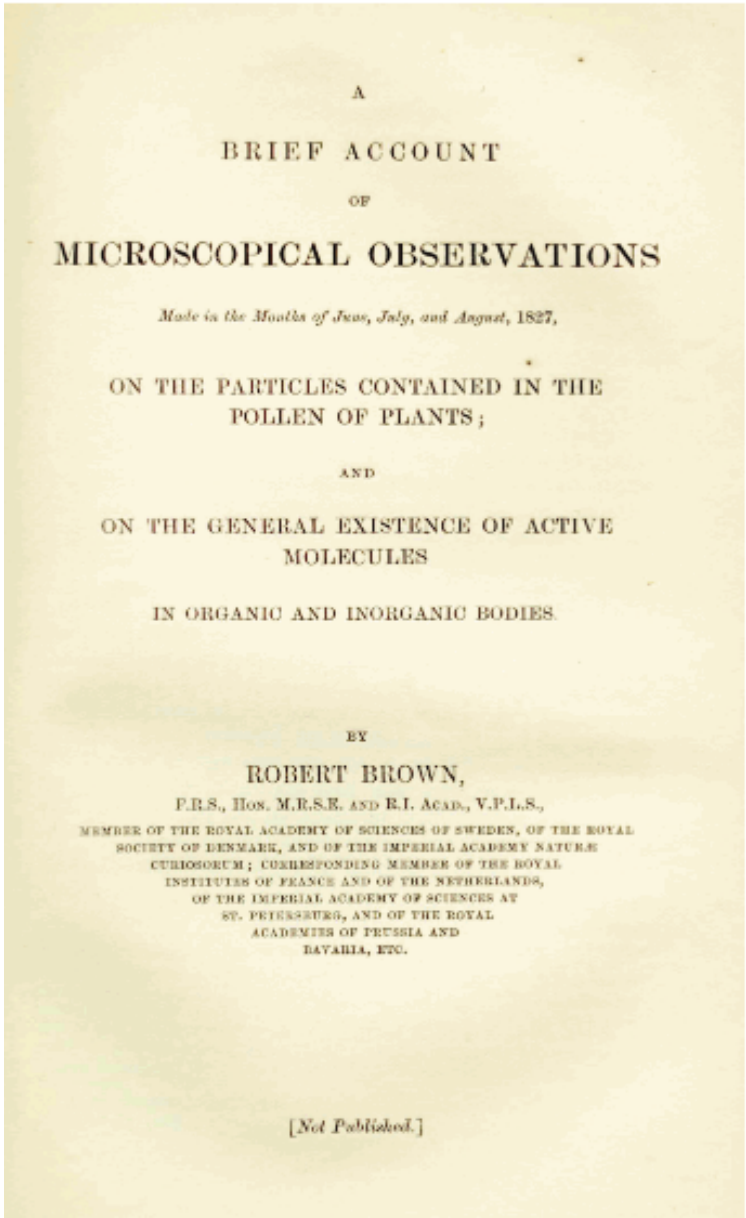


Figure 3-31 Essential Cell Biology 3/e (© Garland Science 2010)

- ***Cells have a few special tricks for storing and accessing energy.***
- ***ATP hydrolysis releases of order 20 kT of energy (note: depends upon concentrations of reactants and products).***

THERMAL ENERGY

- ***Microscopic particles have an intrinsic energy as a result of their contact with the surrounding medium (thermal reservoir).***
- ***k_BT is the thermal energy scale – 4.1 pN nm.***



A

BRIEF ACCOUNT

OF

MICROSCOPICAL OBSERVATIONS

Made in the Months of June, July, and August, 1827,

ON THE PARTICLES CONTAINED IN THE POLLEN OF PLANTS;

AND

ON THE GENERAL EXISTENCE OF ACTIVE MOLECULES

IN ORGANIC AND INORGANIC BODIES

BY

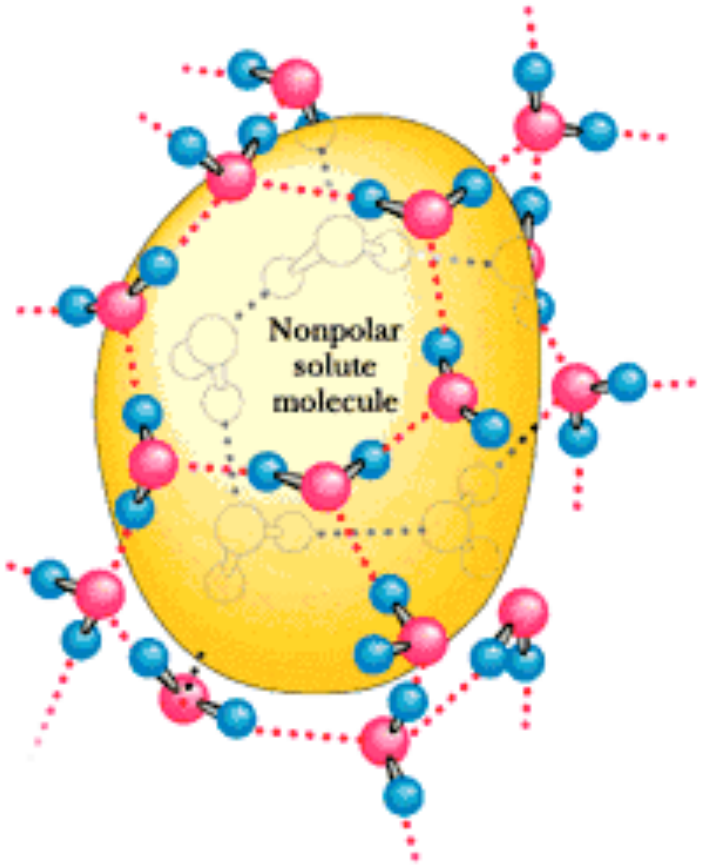
ROBERT BROWN,

F.R.S., Hon. M.R.S.E. and R.I. Acad., V.P.L.S.,

MEMBER OF THE ROYAL ACADEMY OF SCIENCES OF SWEDEN, OF THE ROYAL SOCIETY OF DENMARK, AND OF THE IMPERIAL ACADEMY NATURÆ CURIOSORUM; CORRESPONDING MEMBER OF THE ROYAL INSTITUTES OF FRANCE AND OF THE NETHERLANDS, OF THE IMPERIAL ACADEMY OF SCIENCES AT ST. PETERSBURG, AND OF THE ROYAL ACADEMIES OF PRUSSIA AND BAVARIA, ETC.

[*Not Published.*]

WATER AND HYDROPHOBICITY



- ***Key point is a broader message related to thermal energies: entropic contributions to the free energy are hiding everywhere.***
- ***See HW2 for estimates on this effect.***

PHOTOSYNTHESIS AND RESPIRATION

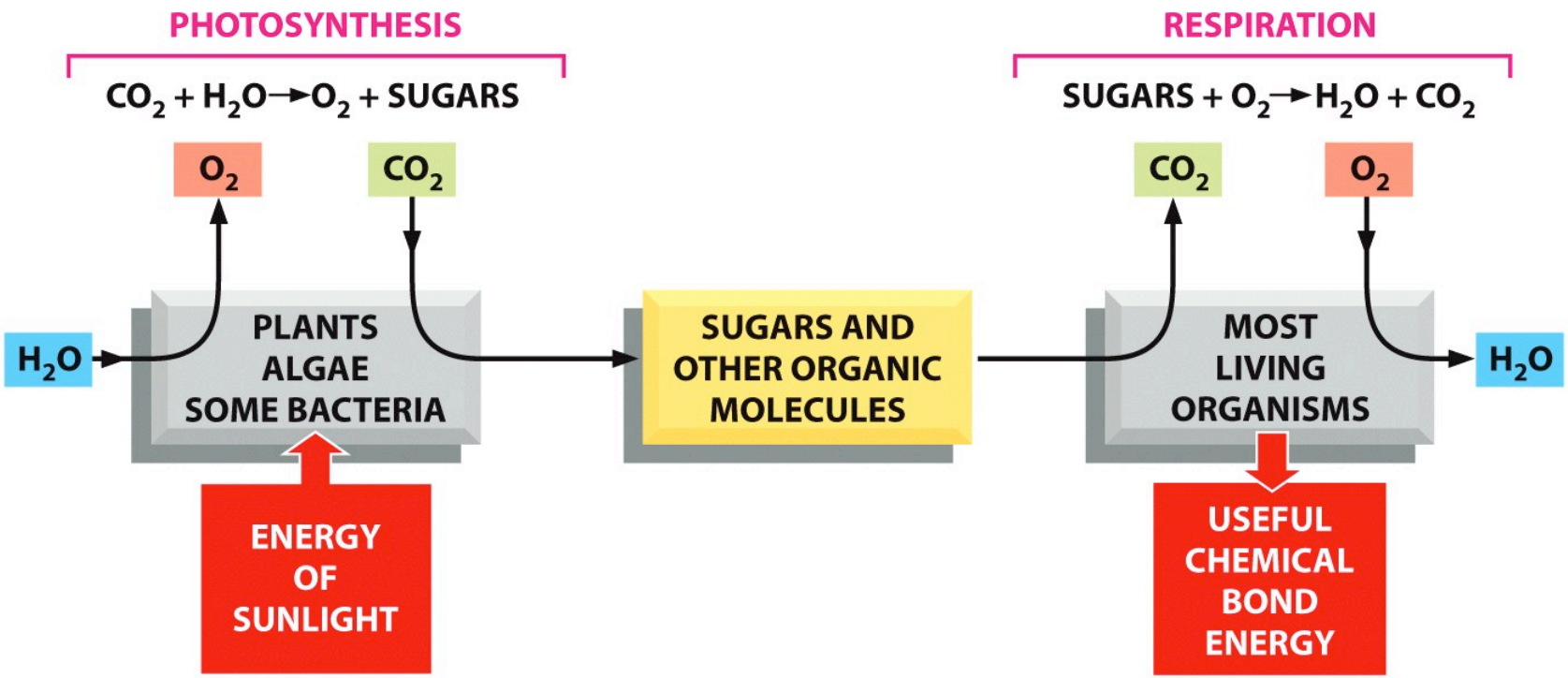
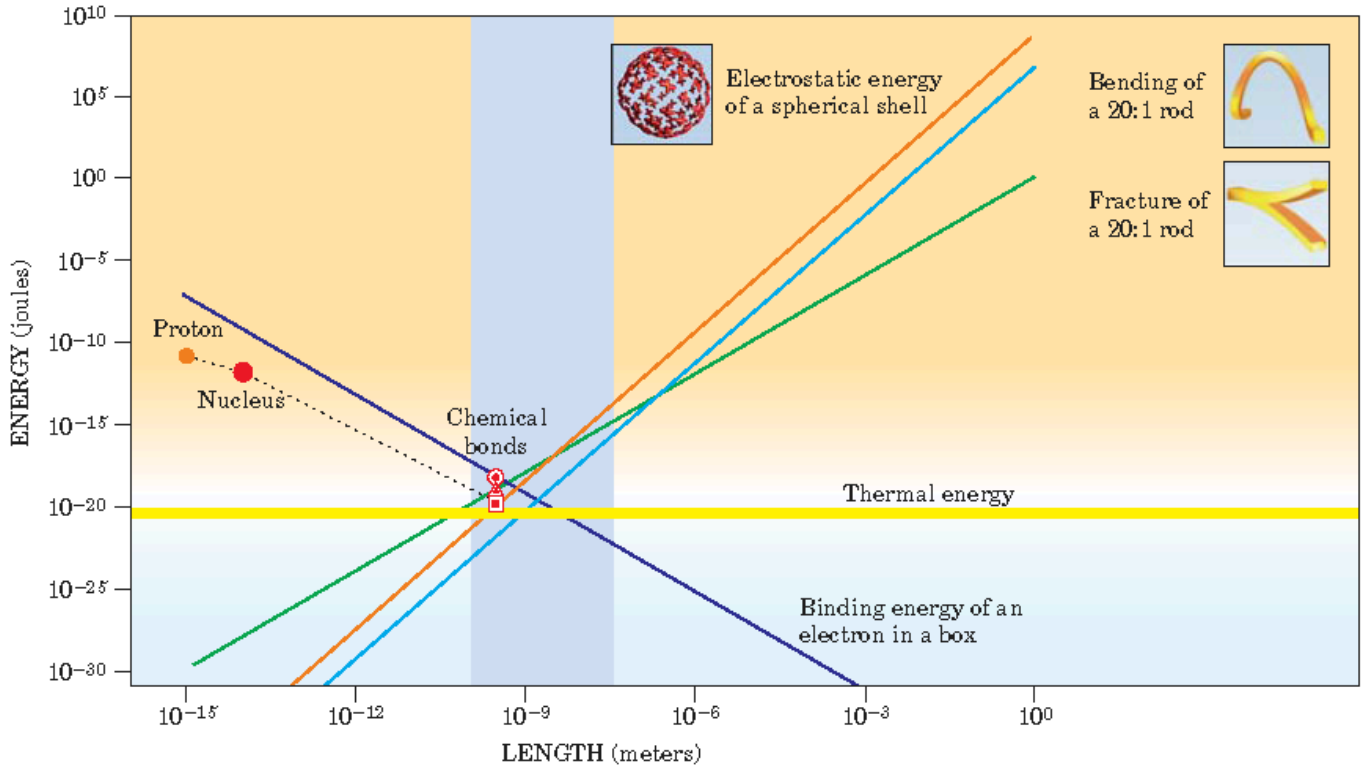


Figure 3-9 Essential Cell Biology 3/e (© Garland Science 2010)

- ***Energy scale of photons: see HW2.***
- ***Energy scale of combustion of a sugar: see HW2.***

A CONFLUENCE OF ENERGY SCALES



- ***Mechanics is played out in a situation where thermal forces are equal partners with their more familiar deterministic counterparts.***
- ***Thermal energy scale – 4.1 pN nm!***

ENERGY AND FORCE SCALES IN BIOLOGY

- ***Energy and force are central themes in biology, just like they are most everywhere else in science and engineering.***
- ***My focus will only be on cells and the molecules within them.***

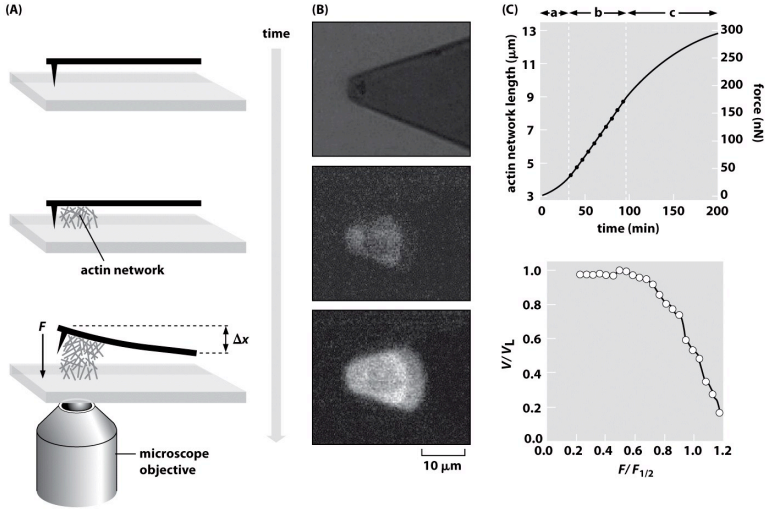
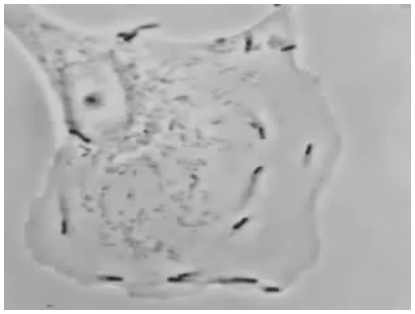


Figure 16.49 Physical Biology of the Cell (© Garland Science 2009)



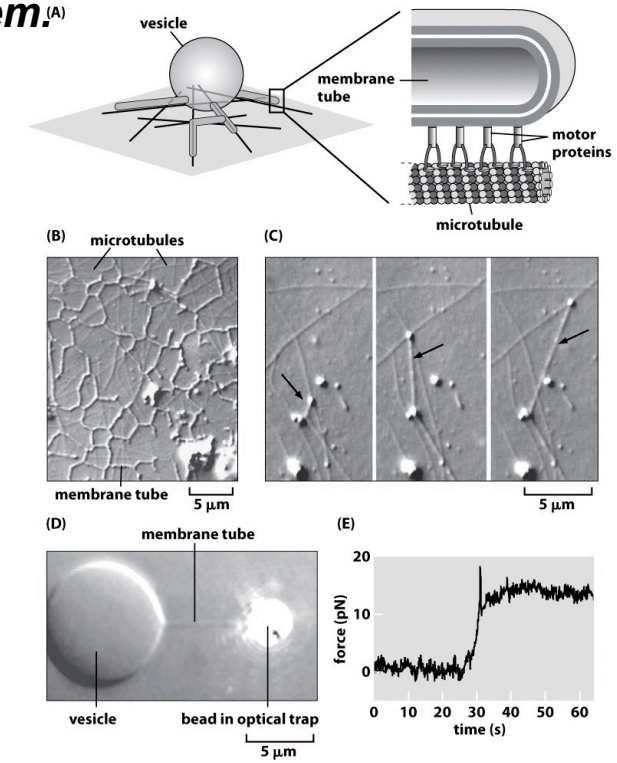
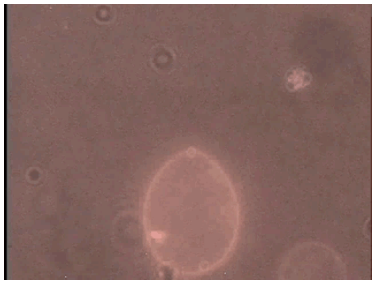


Figure 11.25 Physical Biology of the Cell (© Garland Science 2009)

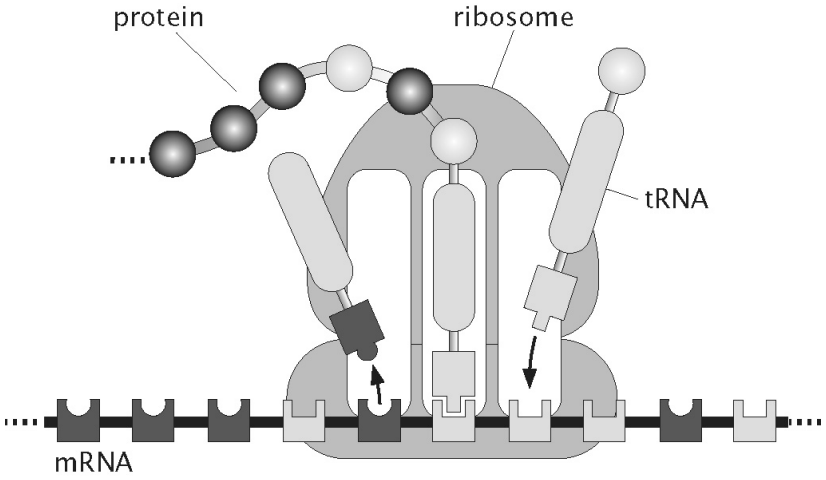


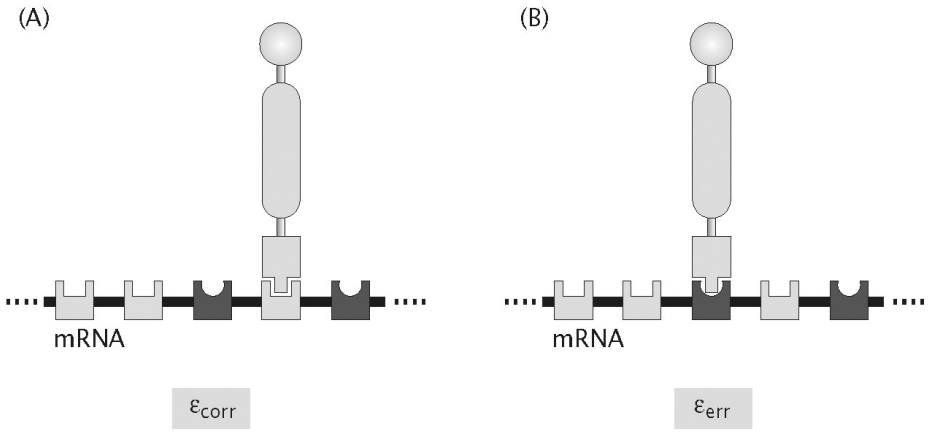
LOST IN TRANSLATION

- ***This example: deterministic and thermal put together.***
- ***Measured error rates of 10^{-4}.***
- ***Correct and incorrect codons can differ by only a single hydrogen bond worth several kT of energy.***

$$error\ rate = \frac{rp_{err}}{rp_{err} + rp_{corr}}$$

$$\approx e^{-\beta\Delta\varepsilon}$$





Key point: the thermodynamic picture of specificity flunks the quantitative sanity check.

A FEELING FOR THE NUMBERS: SUMMARY

- ***Barbara McClintock insisted on developing a "feeling for the organism".***
- ***Our thrust in this course is to use biological numeracy as our vehicle for developing such a feeling for the organism.***
- ***So far, we have explored five big themes: sizes, concentrations, rates, information and energy.***
- ***With this intuitive foundation in hand, we now turn to the main business of the course which is the physical dissection of an array of interesting biological mechanisms.***