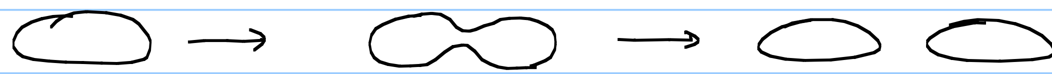
Lecture 2: Stopwatches of many scales

Note Title 7/2/2007

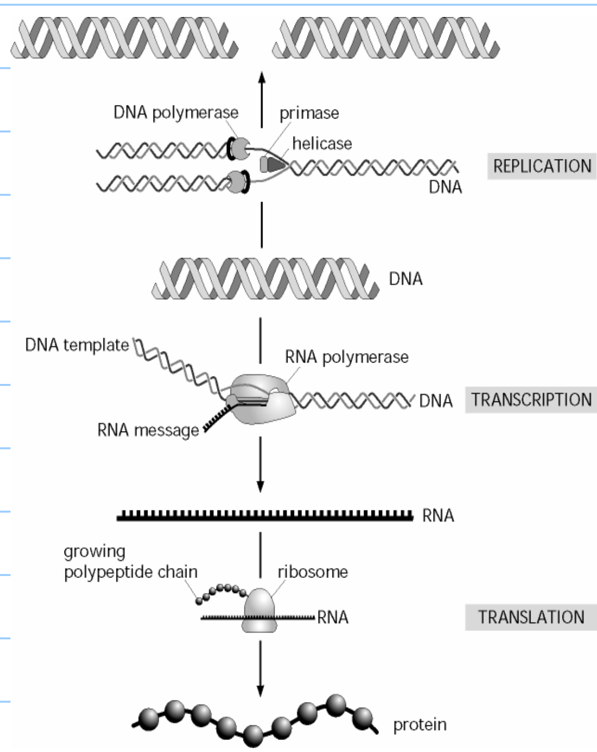
a. Timing of the central dogma

Bacterial cell division $T \simeq 3000$ sec



Cell division time places constraints on the timing of the processes of the central dogma.

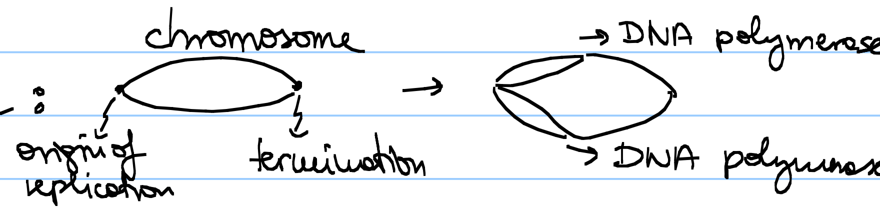
Idea: During the time for one cell division, the cell content is duplicated.



1. Rate of DNA replication

$$\frac{\Delta N_{bp}}{\Delta t} = \frac{5\times10^6\ bp}{3{,}000\ s} = 1.3\times10^3\ bp/s$$

E. coli genome :



We conclude that the DNA polymerases add base-pairs at $\approx \frac{1300}{2}\ \frac{bp}{s} = 650\ bp/s$.
(Biochemical studies find rates $\approx$ 250 - 1000 bp/s)

2. Rate of translation

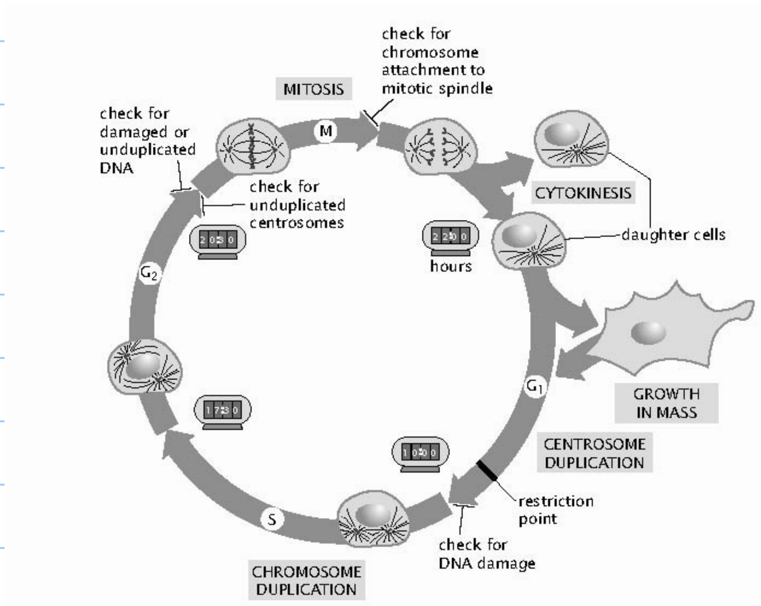
Cell contains $3\cdot10^6$ proteins. Assuming a typical protein is 300 amino acids in size we arrive at :

$$\frac{\Delta N_{aa}}{\Delta t} = \frac{3\cdot10^6\times300\ aa}{3000\ s} = 3\cdot10^5\ \frac{aa}{s}$$

Given 15,000 ribosomes on which protein synthesis takes place we conclude that each polymerase adds

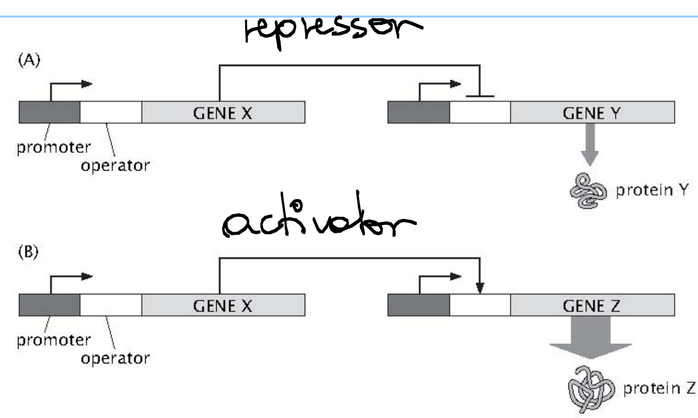
$$\frac{300{,}000\ aa/s}{15{,}000} = 20\ aa/s$$ (25 aa/s is the measured value

b. Cells manipulate time



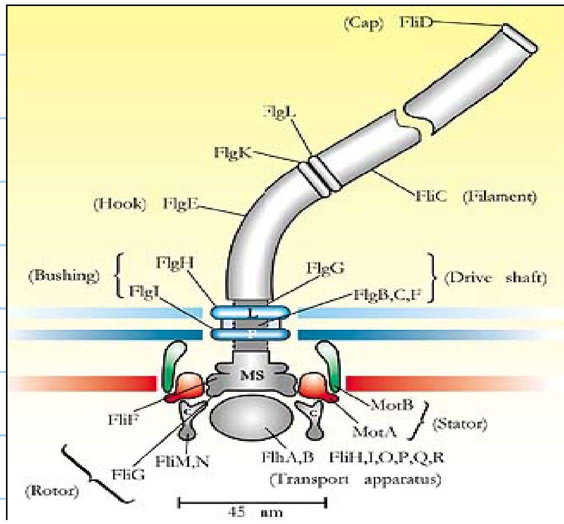
Cell cycle of a typical animal cell.

The timing of the cell cycle is regulated by a variety of checkpoints which, if not satisfied, will prevent the cell from starting the next phase of the cell cycle.



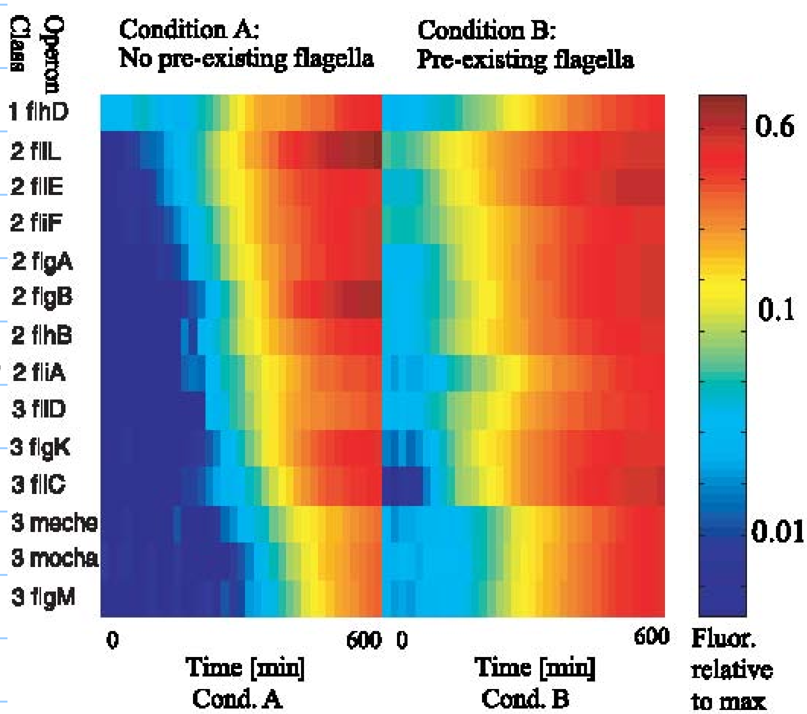
Regulation of gene expression. Protein X either represses or activates the expression of protein Y or Z.

Assembly of the bacterial flagellum is precisely orchestrated in time.



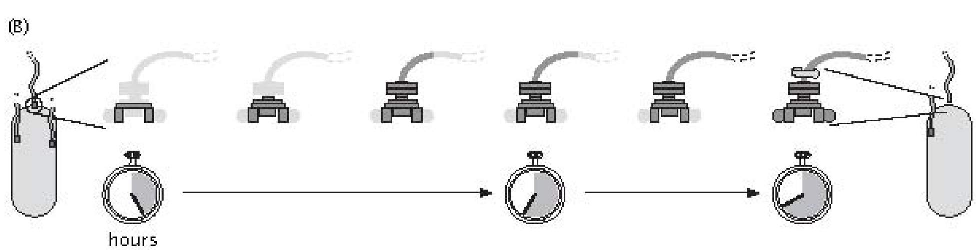
activates class 2 genes

activates class 3 genes



Kalir et al., Science 292, 2080 (2001)

Different strains have GFP reporting on the promoters for different parts of the flagellum.

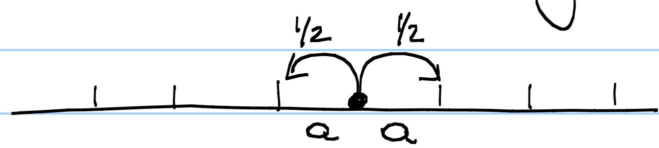


c. Diffusive transport

Molecules left to their own devices will diffuse in solution. For situations when diffusion is too slow, evolution has invented active transport mechanisms.

Random walk model of diffusion

Δt:



$N = \frac{t}{\Delta t}$ steps leads to total displacement

$$\Delta X = e_1 a + e_2 a + \dots + e_N a$$

e_i's are random numbers independently distributed; $e_i = \begin{cases} +1 \\ -1 \end{cases}$ with equal probability

$$\langle \Delta X \rangle = a \left[\langle e_1 \rangle + \langle e_2 \rangle + \dots + \langle e_N \rangle \right]$$

$$= N a \langle e \rangle \qquad \langle e \rangle = +1 \cdot \tfrac{1}{2} + (-1) \tfrac{1}{2} = 0$$

$$= \phi$$

$$\langle \Delta X^2 \rangle = a^2 \left[\langle e_1^2 \rangle + \langle e_2^2 \rangle + \dots + \langle e_N^2 \rangle \right.$$
$$\left. + 2 \langle e_1 e_2 \rangle + 2 \langle e_1 e_3 \rangle + \dots \right]$$

$$\langle e_i^2 \rangle = \frac{1}{2} 1 + \frac{1}{2} 1 = 1$$

$$\langle e_i e_j \rangle = \frac{1}{4} [\underbrace{(+1)\cdot(+1)}_{1}] + \frac{1}{4} [\underbrace{(+1)(-1)}_{-1}] + \frac{1}{4} [\underbrace{(-1)(+1)}_{-1}] + \frac{1}{4} [\underbrace{(-1)\cdot(-1)}_{-1}] = \phi$$

$$\Rightarrow \quad \langle \Delta X^2 \rangle = a^2 N = a^2 t/\delta t = 2Dt$$

($D \equiv \frac{a^2}{2\delta t}$ is the diffusion constant for our RW model)

To estimate D for molecules of various size we revert to the Einstein-Stokes formula

$$D = \frac{k_B T}{\gamma} \qquad \gamma = \text{friction coefficient}$$

$\gamma = 6\pi\eta R$ for ⊖ R

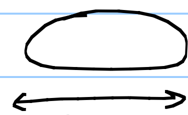
η: viscosity of surrounding fluid

($\eta = 10^{-3}$ Pa s for water)

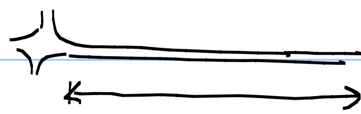
Typical protein in water: $D \simeq \dfrac{4 \times 10^{-21}\,\text{Nm}}{6 \cdot 3 \cdot 10^{-3} \frac{\text{N}}{\text{m}^2}\text{s} \cdot 2.5 \times 10^{-9}\,\text{m}}$

$D \approx 10^{-10}\,\text{m}^2/\text{s} = 100\,\mu\text{m}^2/\text{s}$ (cfr. Bicoid in Drosophila embryo $D \simeq 1\,\mu\text{m}^2/\text{s}$.)

Diffusion time for typical protein across ...

... E. coli  2 μm

$$t_{E.coli} \simeq \frac{(2\,\mu m)^2}{2 \cdot 100\,\mu m^2/s} = 20\ ms$$

... axon 

10 cm = 10^{-1} m = 10^5 μm

$$t_{axon} \simeq \frac{(10^5\,\mu m)^2}{2 \cdot 10^2\,\mu m^2/s} \simeq 5 \cdot 10^{13}\,s$$

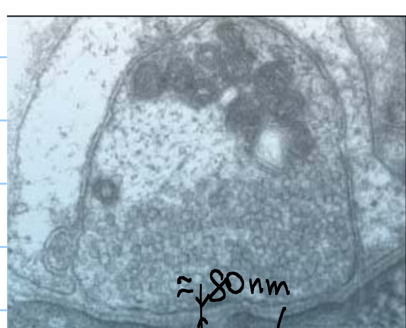
$\simeq$ 1.5 years !

Proteins do not diffuse along axons but are instead shuttled by kinesin moving along microtubules.

Speed of kinesin on microtubules: $v_K \approx 1\,\mu m/s$

$$\Rightarrow t_{axon}^{kinesin} \simeq \frac{10^5\,\mu m}{1\,\mu m/s} \simeq 10^5\,s \simeq 30\ hrs$$

big improvement over diffusion



synoptic cleft

Diffusion of ACh across synoptic cleft of the neuromuscular junction

$$D_{ACh} = D_{protein} \frac{R_{protein}}{R_{ACh}} = D_{protein} \left(\frac{M_{protein}}{M_{ACh}}\right)^{1/3}$$

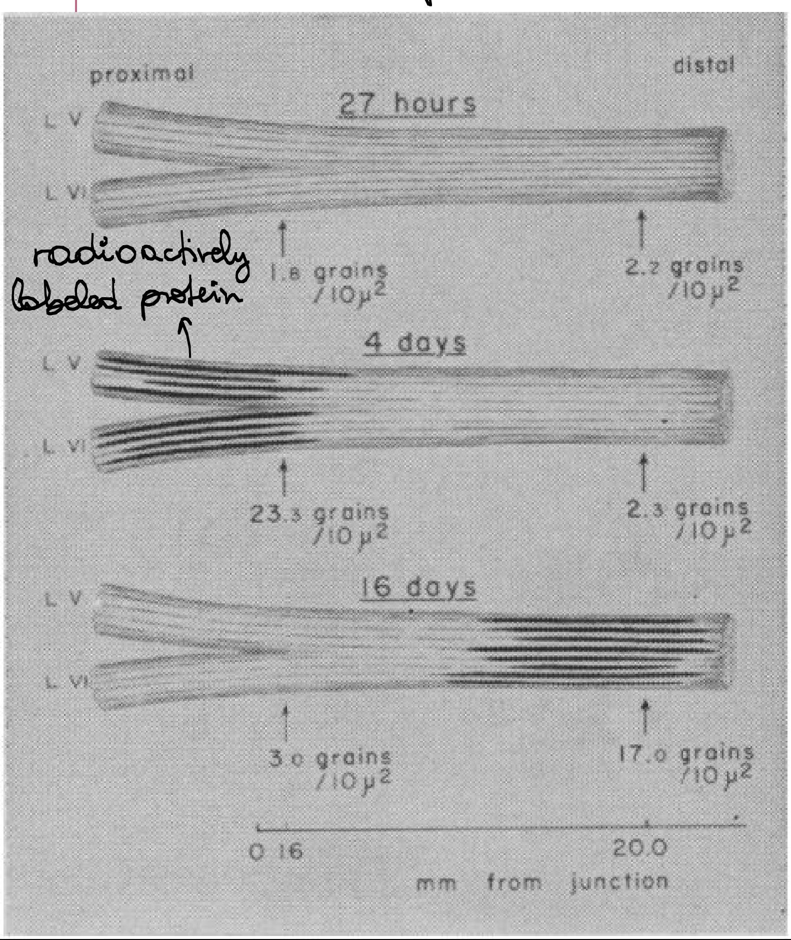
$$D_{ACh} \simeq 100\,\frac{\mu m^2}{s} \left(\frac{30{,}000\,Da}{500\,Da}\right)^{1/3} \simeq 400\,\frac{\mu m^2}{s}$$

$$t_{synaps} \simeq \frac{(80\,nm)^2}{2 \cdot 400\,\mu m^2/s} = 8\,\mu s.$$

Compare this to time for action potential propagation from brain to fingers : $t_{action\,pot} = \frac{1m}{\sim 30\, m/s} = 30\, ms$

speed of action pot.

Axonal transport :



→ Evidence of motors at work ?

$\Delta X = 18\, mm$

$$t_D \approx \frac{\Delta x^2}{2D} \approx \frac{(18 \times 10^{-3}\, m)^2}{2 \cdot 10^{-10}\, m^2/s} \simeq 1{,}6 \times 10^6\, s \simeq 20 \text{ days !}$$

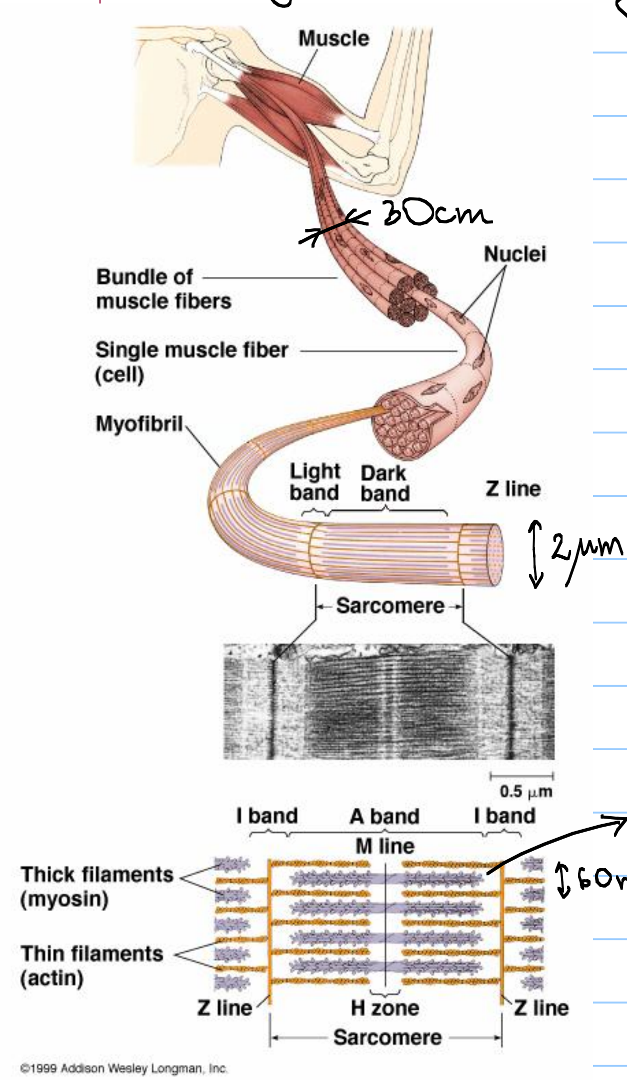
Estimate of average speed of axonal transport

$$v \simeq \frac{18\, mm}{12\, days} \simeq \frac{18 \cdot 10^{-3}\, m}{12 \cdot 3600 \cdot 24\, s} \simeq 2 \cdot 10^{-8}\, m/s = 20\, \frac{nm}{s}$$

Force and energy

$$1 k_B T = 4\,\mathrm{pN\,nm} \quad \begin{cases} \text{Force scale of mol. motors} \sim \mathrm{pN} \\ \text{Displacement} \sim \mathrm{nm} \end{cases}$$

a. Myosin walking on actin



≈ 300 myosins

30cm : muscle fiber

In a cross section of a muscle cell there are:

$$N_{myosin} = \frac{(30\,\mathrm{cm})^2}{(60\,\mathrm{nm})^2} \times 300 \simeq 10^{14}$$

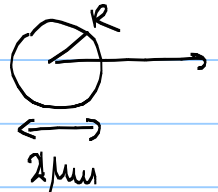
myosin motors.

If the myosins support a weight of $10\,\mathrm{kg} \cdot g \simeq 100\,\mathrm{N}$

then each myosin exerts

$$F_{myosin} \simeq \frac{10^2}{10^{14}}\,\mathrm{N} \simeq \underline{\underline{1\,\mathrm{pN}}}$$

b. Swimming E. coli



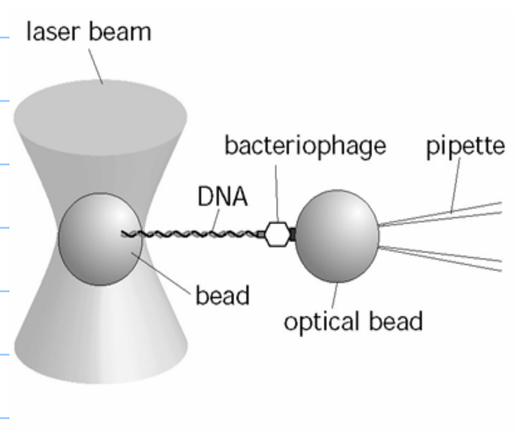
$v = 20\,\mu m/s$

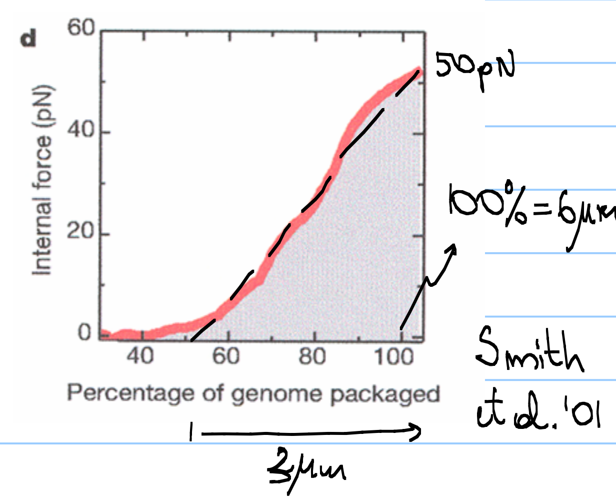
Stokes drag: $F = 6\pi\eta R v$

$$F = 6 \cdot 3 \cdot 10^{-3}\ 10^{-6}\ 20\times10^{-6}\ N$$

$$F = 400\times10^{-15}\,N = 0.4\,pN$$

c. Viral assembly





Smith et al. '01

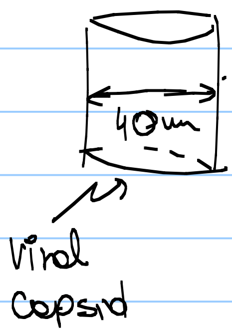
Energy cost of packing genome: $\Delta G_{pack} = \dfrac{50\,pN \cdot 3\,\mu m}{2}$

$= 75\,pN\,\mu m$

$\Delta G_{pack} = 75\times10^{3}\ \mathrm{pN\,nm} \simeq 20{,}000\ k_BT$

What are the physical origins of this energy?

- DNA bending

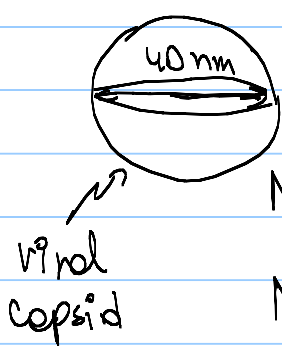


$R_{typ} \approx 10\ \mathrm{nm}$: typical radius of curvature

$$E_{bend} = \frac{\xi_p k_BT}{2}\frac{L}{R_{typ}^2} = \frac{\cancel{50}}{2}\times\frac{6{,}000}{\cancel{100}\,2}\,k_BT$$

$$= 1{,}500\ k_BT$$

- DNA charge



$$E_{charge} = \frac{1}{4\pi\varepsilon_0 D}\frac{Q^2}{R} = \underbrace{\frac{e^2}{4\pi\varepsilon_0 D k_BT}}_{l_B \approx 0.7\,\mathrm{nm}}\frac{N^2}{R}\,k_BT$$

$N = N_{bp}\times 2$

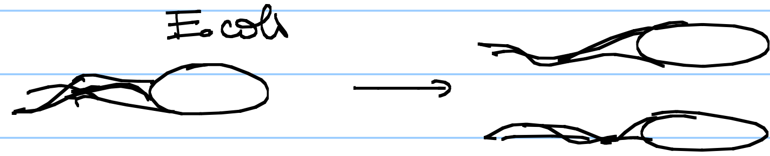
$N = 6\,\mu\mathrm{m}\cdot 3\,\frac{\mathrm{bp}}{\mathrm{nm}}\times 2 = 40{,}000$

$$E_{charge} = 16\times10^{8}\,\frac{0.7}{20}\,k_BT$$

$$= 6\times10^{7}\ k_BT\ !$$

What gives? (Need to consider screened Coulomb...

d. Energy cost of making offspring



Class	Biosynthetic Cost (aerobic) - ATP equiv.
protein	1.2×10^{10}
DNA	3.5×10^{8}
RNA	1.6×10^{9}
phospholipid	3.2×10^{9}
lipopolysaccharide	3.8×10^{8}
peptidoglycan	1.7×10^{8}
glycogen	3.1×10^{7}