BIOREPS Problem Set #10

Of Protein Carts and DNA Railroads: Helicase Unwinding of DNA

1 Introduction

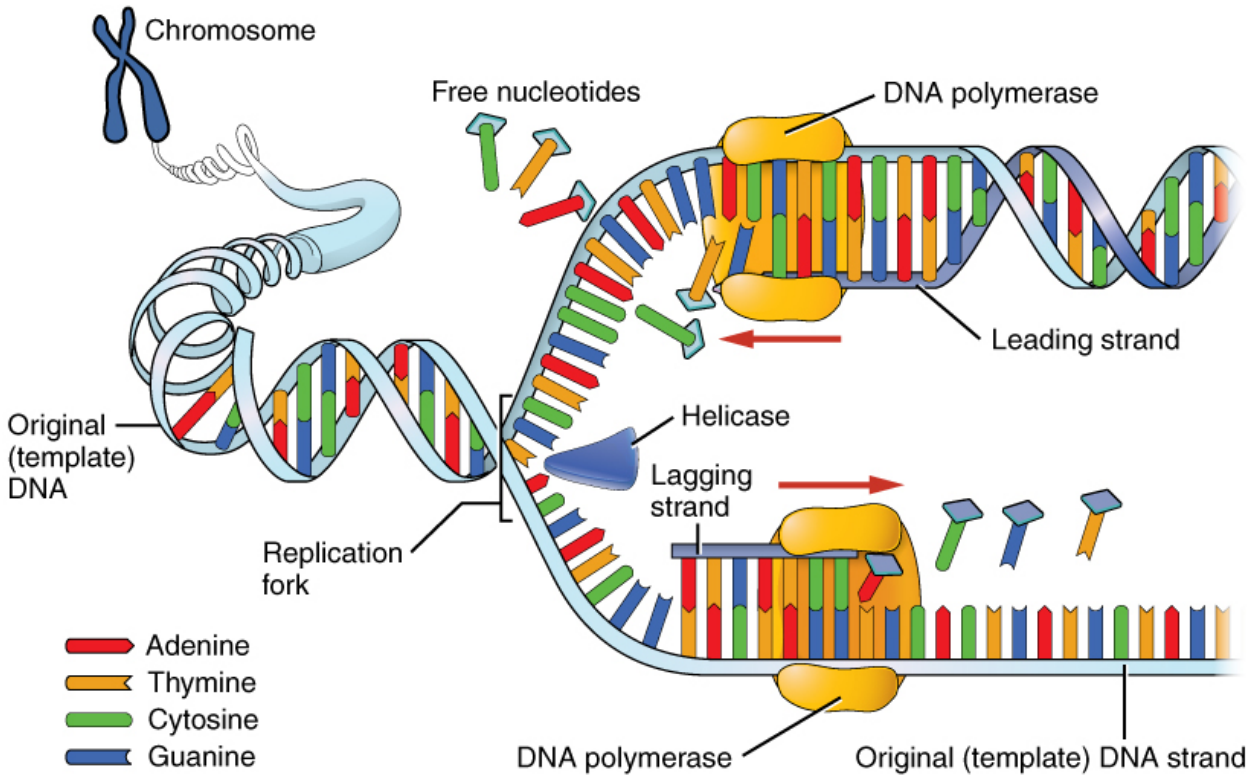

Figure 1: A schematic of the process of DNA replication

The biological world is hallmarked by the singularly critical process of proliferation- the ability to generate progeny capable of maintaining homeostasis and carrying out the multitude of physiological processes that mark the species including further reproduction. The building blocks in all living organisms - cells - must then be capable of duplication. The process of cell division is guided by the need to preserve functionality and sustenance in both the mother and the daughter cell. The progenitor and the progeny thus both need to be equipped with their own sets of genetic material leading to the need for *DNA replication*. Nature has in place stunningly sophisticated cellular micro-architecture to ensure this replication of a cell's genome during cell division giving rise to two self-contained, complete units.

A DNA molecule is made up of two individual strands, each with a sugar-phosphate backbone on which are mounted the nucleobases. The two strands are linked by hydrogen bonds between the complementary bases. This assignment deals with the first step in this replication process:

the "unzipping" of the parent molecule (hereby referred to as dsDNA-double stranded DNA) into its two constituent strands (ssDNA- single stranded DNA) by the rupture of the hydrogen bonds between the base pairs. A special kind of motor protein carries out this task: the helicase protein. ssDNA are polar molecules, with an inherent directionality defined by the exposed sugar phosphate backbone. Helicase proteins are capable of utilizing this electric gradient to propel themselves along the ssDNA strand. Along the way, they might encounter obstacles in the form of zipped segments (two complementary or neighboring ssDNA strands linked to form an ss-ds junction). The helicase can transduce and channel energy from ATP hydrolyses to move one ssDNA relative to the other, causing a rupturing of the junctions and forming an ′unzipped′ chain. It then moves along this new added segment to its track and continues on. The problem can be summed up in a line as: motion of a motor protein ′cart′ on a mobile DNA ′railroad′.

2 Background Model: the Helicase-DNA System

This assignment closely follows the treatment from a 2003 study on the working principles of a general bio-motor advancing against a mobile obstacle [1], in which helicase unwinding of DNA has been chosen as a particulary illustrative example. The position of the motor protein helicase, indicated by the nucleobase to which it is bound, is labelled by the integer index *n*. The ss-ds ′obstacle′ junction is labelled by the integer *m*.

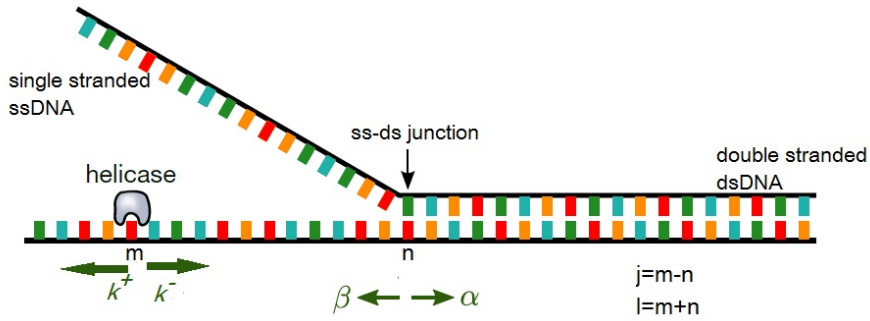

Figure 2: A Schematic of the Helicase-DNA unwinding system. *Image courtesy: The Stat May Qual Question β, CWRU Physics, 2015*

The interaction between the helicase and the ss-ds junction that leads to subsequent unzipping can be characterized by an interaction potential *U(m-n)*, reasonably assumed to depend only on the helicase-junction separation along the strand. The simplest possible choice for U(m-n) would be a hard wall potential $U = 0$ for $m > n$, and $U \to \infty$ for $m \leq n$. This would completely deter the helicase from moving on the DNA beyond the junction. In this case, further motion is only possible if some fluctuation in m can reverse the inequality. This could be facilitated by thermal oscillations (the ertswhile ′Brownian ratchet′ [2]) or other ways in which fluctuations can be induced in externally applied energy fields ([3],[4] and [5]). A far more realistic model for biological systems like the motor protein system here would be softer potential with a finite range. When the helicase advances to the ss-ds junction, the interaction between the helicase and the junction acts as a catalyst that affects not just the motion of the helicase, but also the kinetics of the the ′unzipping′. Following our reference [1], we will derive analytical expressions for the speed of unwinding and how it is affected by the interaction strength, as well as discuss various biologically-relevant limits on such speeds and the enzymatic effect the presence of the helicase at the junction has on this process.

Figure 3: An artist's impression of Helicase unwinding a DNA strand. *Source: Nature Scitable, "Cells Can Replicate Their DNA Precisely"*

References

[1] A Motor that Makes Its Own Track: Helicase Unwinding of DNA *Phys. Rev. Lett.* **25**, 81031–81034 (2003).

[2] Cellular motions and thermal fluctuations: the Brownian ratchet *Biophys.J* **65**, 316 (1993)

[3] Brownian ratchets in physics and biology *Contemp.Phys.* **38**, 371-379 (1997)

[4] Influencing intramolecular motion with an alternating electric field *Nature* **406**, 608-611 (2011)

[5] Directing cell motions on micropatterned ratchets *Nat.Phys.* **5**, 606-612 (2009)

3 Questions

3.1 Interaction-dependent Rates

For the ease of analysis and in keeping with the coarse-grained nature of the available experimental data, we will design a discrete system where both the helicase and the ss-ds junction can only move in finite jump steps. We denote the forward and backward hopping rates of the helicase protein (away from the ss-ds junction) by k^+ and k^-, respectively. The corresponding forward and backward rates for the junction are α and β. If *E* is the free energy difference per base between the dsDNA and its two constituent ssdNA strands, detailed balance would then immediately dictate $\alpha/\beta = e^{-E/k_BT}$. On a similar note, we can state that $k^+/k^- = e^{\Delta\mu/k_BT}$, where $\Delta\mu$ is the chemical free energy of ATP hydrolysis (i.e the fuel for the motion of the helicase cart).

a) The interaction between the helicase and the ss-ds junction is now given by the earlier introduced $U(m-n)$. Using the above information, justify with appropriate reasoning how the interaction-dependent hopping rates will now obey:

$$\frac{\beta_j}{\alpha_{j-1}} = \frac{\beta}{\alpha} e^{-[U(j-1)-U(j)]/k_BT} \tag{1}$$

$$\frac{k_j^+}{k_{j-1}^-} = \frac{k^+}{k_-} e^{-[U(j-1)-U(j)]/k_BT} \tag{2}$$

α_j, β_j, k_j^+ and k_j^- are now the position-dependent rates, and $j = m-n$ is the helicase-junction separation index.
Hint: The interaction will introduce new terms in the system's Hamiltonian.

Equations (1-2) show us that for our initial consideration of a hard wall potential, $\beta_1 = 0$ at the wall, but also $k_1^+ = 0$. So while the helicase is able to prevent the ss-ds junction from closing in further beyond $j = 1$ (or $m = n + 1$), it also is not able to move and can do precious more than being a guard-dog. To make things more interesting, we soften the interaction potential and lend it a finite range. We will see how this now allows for the helicase to participate in enzymatically-assisted unwinding of the DNA.

3.2 Mastering the Master Equation

To preserve the simple discrete nature of the system, we impart a finite range to the interaction potential in discrete steps of N, each of strength U_0 (Fig.2). This now allows the helicase to take a finite N number of steps beyond $m = n$ before it reaches the junction, given it can keep counteracting the obstacle potential steps. The helicase can use energy from ATP hydrolysis to further influence the speed of the junction, causing it to split the DNA faster. However, this energy of interaction also acts as a check on the helicase motion, preventing it from progressing along the DNA chain too quickly. This trade-off thus leads to an optimum situation for unwinding.

a) Having defined the local rates, let us label the state of the system at any time t by the numbers j and l; where $j = (m - n)$ and $l = (m + n)$. j determines the interaction potential, while the center of motion variable l incorporates the mobility of the ss-ds junction itself. Show that for each hopping time step δt, the system can change in only one of four ways. What are the corresponding transition probabilities?
Hint: Think of all the possible combinations, the displacement can happen in this scenario, in terms of j and l.

b) Put the above results together to show that the master equation for $P(j, l, t)$ becomes:

$$\begin{aligned}\frac{\partial P(j,l)}{\partial t} = &-(\alpha_j + \beta_j + k_j^+ + k_j^-)P(j,l) + \alpha_{j-1}P(j-1,l-1) + \\ &\beta_{j+1}P(j+1,l+1) + k_{j+1}^+P(j+1,l-1) + k_{j-1}^-P(j-1,l+1)\end{aligned} \tag{3}$$

Earlier, we saw how the hopping rates depend only on j. With this prior knowledge, it would make sense to eliminate l by summing over to obtain $P_j = \sum_l P(j, l)$. This j dependent probability relaxes to a stationary state distribution with a characteristic time scale determined by the free state hopping rates α, β, k^+ and k^-.

The interaction potential is constrained by i) $U \to 0$ for $j \to 0$ and ii) $U \to \infty$ for $j \to \infty$. These limits ensure physically realistic conditions of no interaction for large separations, as well as always ensure the helicase is located on the ssDNA strand. The relevant regime of interest is thus in the locality of the ss-ds junction.

c) Noting that the potential blows up as $j \to -\infty$, show that the stationary state solution can be described by the recursion relation:

$$\mathcal{P}_{j+1}^s = \frac{k_j^- + \alpha_j}{k_{j+1}^+ + \beta_{j+1}}\mathcal{P}_j^s \tag{4}$$

3.3 Unzipping Rates

a) Using the appropriate transition/ unwinding rates and the probability of finding the helicase-junction system at the corresponding separations, we can obtain an expression for the the effective unwinding rate at the difference variable j in terms of k_j^+, α_j, k_j^- and β_j. Show that the expression for mean velocity (bp/ sec) of DNA opening can be written as:

$$v = \frac{1}{2}\sum_j (k_j^+ + \alpha_j - k_j^- - \beta_j)\mathcal{P}_j \tag{5}$$

Make note of the fact that there two directions the ss-ds junction position can move in.

b) If now a hard wall potential (i.e $N = 0$) at $j = 0$ is considered, then $k_1^+ = \beta_1 = 0$. The probability distribution for $j > 0$ can now be taken in the form $P_j = Ac^j$, where A is a normalization

constant and $c = (\alpha + k^-)/(\beta + k^+)$. Show how the average velocity for the simple hard wall case becomes:

$$v_{avg,HW} = \frac{\alpha k^+ - \beta k^-}{\beta + k^+} \tag{6}$$

$v_{avg,HW}$ will be positive whenever $k^+/k^- > \beta/\alpha$ is satisfied. This corresponds to whenever the free energy difference E (recall how it is the driver of the unwinding process) is smaller than the chemical free energy gradient from ATP hydrolysis $\Delta\mu$.

Let us now make our first improvement on this scenario, notching N up to $N = 1$, i.e a one step potential. The potential strength is now given by $U_0 \equiv [U_0 - U(j = 1)/k_BT]$. The helicase can then cross and overcome the junction barrier if it can harness enough energy to match this U_0. While we already know the ratio of the hopping rates from Eqns (1-2), the actual values of the rates are determined by the energy gradient between the states $U(j = 1)$ and $U(j = 0)$. While the actual details of incorporating the energy gradient into the interaction energies could be quite involved, a simple way to factor it in and qualitatively study its effect would be via a dimensionless coefficient f where $0 < f < 1$. f is a measure of the strength of the gradient, and the hopping rates can be modified to the forms:

$$k_1^+ = k^+ e^{-fU_0} \tag{7}$$

$$k_0^- = k^- e^{-(f-1)U_0} \tag{8}$$

$$\beta_1 = \beta e^{-fU_0} \tag{9}$$

$$\alpha_0 = \alpha e^{-(f-1)U_0} \tag{10}$$

c) We can now study the dependence of the velocity on this interaction strength gradient. Denoting the new average velocity by v_1, it can be shown that the velocity increase of the one-step potential case relative to the hard wall case will be given by,

$$\frac{v_1}{v_{HW}} = \frac{c + (1 - c)e^{-fU_0}}{c + (1 - c)e^{U_0}} \tag{11}$$

Plot this ratio versus the step height, for some representative values of f, and qualitatively discuss the behaviour of the graphs. What factors could account for/ explain the initial increase and then the decrease of the ratio with increase in step height?

d) From the nature of the unwinding rate versus step height strength plots, it is clear that for any set of parameter values for f and c, there is a maximum unwinding rate obtained at a certain barrier height. Let us denote this barrier height by $U_0 = U_*$. We can now obtain U_* from the relation:

$$fe^{U_*} - e^{fU_*} = \frac{(1 - c)(1 - f)}{c} \tag{12}$$

Use Eqn (12) to show that the one-step potential unwinding rate has an upper bound given by:

$$v_{1,max} = \frac{v_{avg,HW}}{c} = \frac{\alpha k^+ - \beta k^-}{\alpha + k^-} \tag{13}$$

e) Bonus Question:

For $N > 1$, the interaction potential is divided into N identical steps, each of step height U_0. In an identical manner as v_1, we can obtain the ratio of the N-step unwinding rate v_N relative to the hard wall case as,

$$\frac{v_N}{v_{HW}} = \frac{c^N + (1-c)e^{-(f-1)U_0} \sum_{j=1}^{N} c^{N-j} e^{-jU_0}}{c^N + (1-c) \sum_{j=1}^{N} c^{N-j} e^{-jU_0}} \tag{14}$$

It is easy to check this reduces to Eqn (11) for $N = 1$. Show plots of this unwinding rate ratio versus the step strength U_0 for $N = 1$, 3 and 5. You will see how the opening rate starts becoming more and more sensitive to U_0 with increasing N. For large N, the unwinding rate starts approaching $U_* \approx -lnc \approx -ln\frac{\alpha}{\beta}$. Thus we can conclude that the optimal step height tends to the free energy gradient of opening of one base pair. The fastest possible opening will then occur with compatibility between the interaction energy of the helicase-junction unit, and the base-pairing energy of the DNA nucleotide units (which is just what we would expect - so we can now rest easy our model is not completely nonsensical!!)