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Short Communication

RNA polymerase pushing

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ABSTRACT

Molecular motors can exhibit Brownian ratchet or power stroke mechanisms. These mechanistic categories are related to transition state position: An early transition state suggests that chemical energy is stored and then released during the step (stroke) while a late transition state suggests that the release of chemical energy rectifies thermally activated motion that has already occurred (ratchet). Cellular RNA polymerases are thought to be ratchets that can push each other forward to reduce pausing during elongation. Here, by constructing a two-dimensional energy landscape from the individual landscapes of active and backtracked enzymes, we identify a new pushing mechanism which is the result of a saddle trajectory that arises in the two-dimensional energy landscape of interacting enzymes. We show that this mechanism is more effective with an early transition state suggesting that interacting RNAPs might translocate via a power stroke.

1. Introduction

DNA transcription is a ubiquitous process in all biological organisms and serves as the primary link between genetic information and phenotype. Central to this process is the molecular machine RNA polymerase (RNAP) which is responsible for reading the DNA sequence and synthesizing an RNA copy. Numerous mechanistic and structural studies of this motor enzyme have revealed the complex series of molecular reorganizations that occur during a single cycle of NTP binding, NTP hydrolysis, RNA chain elongation and forward translocation along the DNA template [1–7]. A variety of pause states with different characteristic lifetimes and different translocation registers relative to the active configuration interrupt the smooth forward progress of the polymerase [8,9]. Of particular interest is the backtracked state, where the enzyme spontaneously loses track of the 3′ end of the RNA and slides backwards (upstream) along the DNA template [10–13]. This state occurs stochastically in a sequence biased manner, may be induced by physical barriers, error incorporation or the application of opposing force [10,11,14] and results in the creation of a passively diffusing state where the polymerase may no longer use the energy from NTP hydrolysis to bias its motion forwards [15].

One effect that has been suggested to contribute to transcriptional regulatory mechanisms is the interaction between multiple polymerases on the same template [16–18]. In analogy to traffic jams, one might expect that transit rates would decrease as the density of polymerases increases [19]. Contrary to that expectation, biochemical experiments have shown that active polymerases are able to rescue stalled backtracked enzymes in front of them and increase the efficiency of transcription through site-specific DNA binding proteins or paused states both in vitro and in vivo [20–23]. Furthermore, recent work has shown that ribosomes translating behind RNA polymerase in bacteria also function to reduce polymerase pausing [24]. These observations suggest that an active polymerase or ribosome uses its NTP-dependent translocation activity to push a backtracked polymerase forward and speed its recovery. Here, we look closer at this pushing hypothesis and show how different mechanisms of translocation lead to different levels of polymerase pushing.

2. Model of polymerase pushing

We describe the behavior of individual enzymes as resulting from motion on a one-dimensional free energy landscape. The motion of an active polymerase is coupled to NTP hydrolysis so that a single base-pair step results in a lower free energy. A backtracked enzyme behaves as a passive diffusive particle so that a step in either direction is isoenergetic to a first approximation. We thus have two interacting particles, a diffusing one (i.e. the backtracking polymerase) that is trailed by an actively stepping one.

The pushing mechanism can be illustrated by deriving a discrete kinetic model from the continuous free-energy landscape that results from the interaction between the motor (active polymerase) and the

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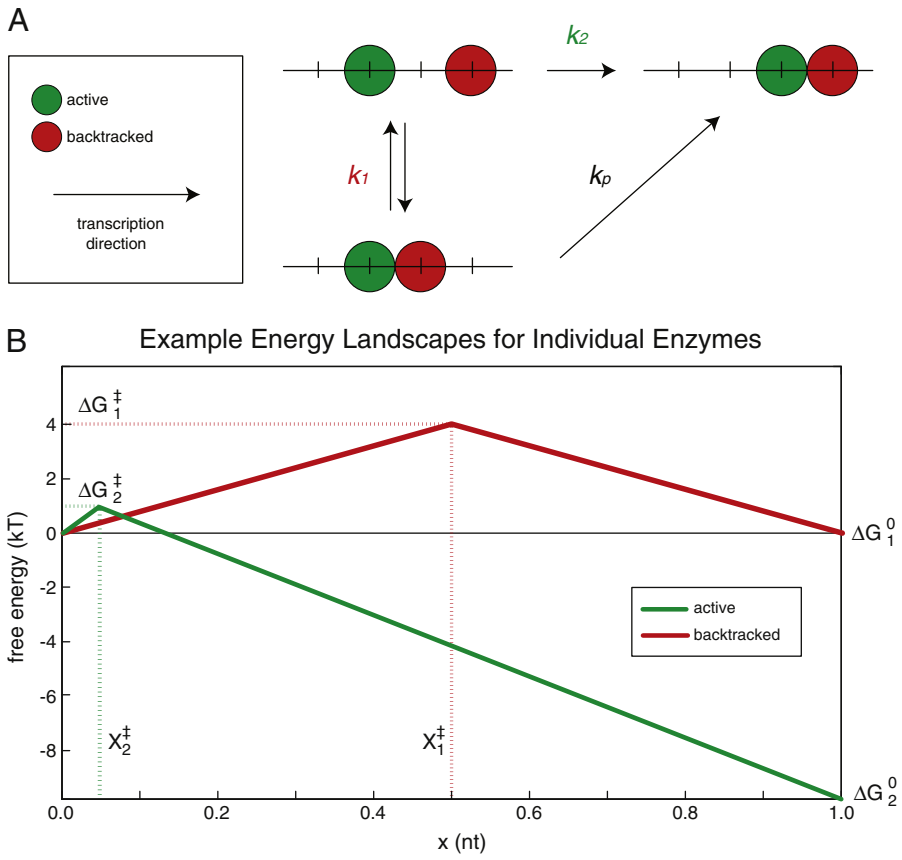

Fig. 1. Model of RNA polymerase pushing. (A) Kinetic scheme of possible moves for two enzymes that have encountered one another on the DNA template where a backtracked enzyme (red) is in front of an active enzyme (green). Two paths that lead to both enzymes stepping forward are shown. (B) An example of the piecewise linear energy landscapes for the stepping of backtracked (red) and active (green) enzymes. Each landscape consists of three parameters: the transition state free energy and position ($\Delta G^{\ddagger}$ and $x^{\ddagger}$) and the step energy (ΔG^{0}).

obstacle (backtracked polymerase). We investigate the two enzyme system shown in Fig.1. Once the enzymes encounter one another, there are two possibilities. The first is shown by the sequential path k_1k_2 where the backtracked enzyme steps forward on its own using energy from the bath (k_1) and then the active enzyme catches up (k_2) (referred to as the passive path). The second possibility is that the active enzyme steps forward and while doing so, pushes the backtracked enzyme forward at the same time (k_P) (referred to as the pushing path). The energy landscapes for each enzyme are constructed in a piecewise linear way. Each individual enzyme landscape is completely determined by three parameters (supplemental information), namely the transition state position ($x^{\ddagger}$), the transition state energy ($\Delta G^{\ddagger}$) and the final energy (ΔG^{0}) where all positions are given in base-pairs and all energies are given in units of kT. We assume that the backtracked enzyme diffuses along an isoenergetic landscape ($\Delta G_1^0 = 0$). Clearly, sequence variations will render this assumption incorrect, but these variations do not affect the conclusions of the analysis nor the essence of the implications for motor interaction and enzyme pushing.

The two enzyme system evolves on a free energy landscape that is given by the sum of the individual landscapes plus an interaction term:

$$G(x_1, x_2) = G_1(x_1) + G_2(x_2) + G_{12}(x_2 - x_1) \quad (1)$$

where x_1 and x_2 denote the positions of each enzyme (Fig. 2A–C). If we assume that enzymes act as hard spheres, $G_{12}(x_2-x_1)$ is zero if $x_1 > x_2 + d$, where d describes the extent of the enzyme, and infinity otherwise. Using a soft repulsive potential with a range that is smaller than the extent of a base-pair does not change the general conclusions that follow. For long ranged potentials other pushing mechanisms have been reported, see discussion below [25–27]. While the system is free to choose any path across the landscape, two limiting cases may be used to compare the two possibilities illustrated in Fig. 1A. Specifically, the backtracked enzyme may take a step forward on its own (vertical gray arrow, Fig. 2A–C) or the active enzyme may take a step forward and push the backtracked enzyme at the same time (diagonal blue arrow, Fig. 2A–C). We then look at the free energy differences along each of these paths (Fig. 2D–F) to estimate relative rates using transition state theory [28,29]. By varying the parameters of the isolated enzyme landscapes (Fig. 1B), we observe how different transition state positions lead to different rates along the pushing path. For example, keeping all other parameters fixed, we vary the position of the transition state for the active enzyme ($x_2^{\ddagger}$) as shown in Fig. 2.

3. Results and discussion

3.1. Enzymes in phase (integer d)

We first assume the extent of a polymerase to amount to an integer multiple of the step size of a base-pair. Three exemplary sets of free energy landscapes are shown in Fig. 2A–C. In Fig. 2D–F, the free energy along the pushing path (blue) is compared to the passive path (gray). The maximum energies along each path are highlighted with colored dots. In this set of parameters, only the landscape with an early transition state position, $x_2^{\ddagger} = 0.2$ (Fig. 2A and D), displays an energy barrier along the pushing path (blue) that is lower than that for the passive motion of the diffusive enzyme alone (gray). The two-dimensional energy landscapes illustrate that a true pushing path (i.e. a saddle trajectory along the diagonal) only exists for $x_2^{\ddagger} < x_1^{\ddagger}$, when the