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Molecular motor translocation kinetics: Application of Monte Carlo computer simulations to determine microscopic kinetic parameters

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ABSTRACT

Methods for studying the translocation of motor proteins along a filament (*e.g.*, nucleic acid and polypeptide) typically monitor the total production of ADP, the arrival/departure of the motor protein at/from a particular location (often one end of the filament), or the dissociation of the motor protein from the filament. The associated kinetic time courses are often analyzed using a simple sequential uniform *n-step* mechanism to estimate the macroscopic kinetic parameters (*e.g.*, translocation rate and processivity) and the microscopic kinetic parameters (*e.g.*, kinetic step-size and the rate constant for the rate-limiting step). These sequential uniform *n-step* mechanisms assume repetition of uniform and irreversible rate-limiting steps of forward motion along the filament. In order to determine how the presence of non-uniform motion (*e.g.*, backward motion, random pauses, or jumping) affects the estimates of parameters obtained from such analyses, we evaluated computer simulated translocation time courses containing non-uniform motion using a simple sequential uniform *n-step* model. By comparing the kinetic parameters estimated from the analysis of the data generated by these simulations with the input parameters of the simulations, we were able to determine which of the kinetic parameters were likely to be over/under estimated due to non-uniform motion of the motor protein.

1. Introduction

Many motor proteins, such as helicases (Lohman et al., 2003, 2008; Pyle, 2008; Patel and Picha, 2000; Matson and Kaiser-Rogers, 1990), polymerases (Kornberg, 2007; Neuman et al., 2003), chromatin remodelers (Becker, 2005; Fischer et al., 2007, 2009), viral DNA packaging motors (Earnshaw and Casjens, 1980; Feiss and Rao, 2012; Yang et al., 2009), polypeptide translocases (Burgess et al., 2016b, 2016; Chatterjee et al., 2013; Lucius et al., 2010; Andrade et al., 2007; Rajendar and Lucius, 2010), some restriction enzymes (Szczelkun, 2002; Firman and Szczelkun, 2000; McClelland et al., 2005; Stanley et al., 2006; Meisel et al., 1995; Crampton et al., 2007), and others (Biebricher et al., 2013; Aussel et al., 2002; Lee et al., 2014; Cattoni et al., 2013) share an ability to translocate processively and with directional bias along a filament (DNA, polypeptide, *etc.*). The translocation of these motor proteins along the filament is coupled with the binding and hydrolysis of nucleotide triphosphates such as adenosine triphosphate (ATP). A variety of techniques/assays have been used to study the translocation process including both ensemble and single molecule experiments (Dillingham et al., 2000, 2002; Brendza et al., 2005; Sikora et al., 2006; Levin et al., 2005; Fischer et al., 2004, 2007, 2009; Kapanidis et al., 2006; Dumont et al., 2006; Galletto and Tomko, 2013; Reuter et al., 2010; Rad and Kowalczykowski, 2012; Nelson et al., 2009; Whitehouse et al., 2003; Saha et al., 2002; Yang et al., 2009; Rajendar and Lucius, 2010; Lucius et al., 2010; Lee et al., 2014). In these experiments the translocation process is typically monitored either by detecting the location of the motor at a particular location along the filament (*e.g.*, the end of the filament) or by measuring the amount of ATP hydrolysis by the translocating motor.

The repeating cycles of nucleotide binding, nucleotide hydrolysis, movement of the motor protein along the filament, and any other associated processes, can be effectively modeled with a sequential uniform *n-step* mechanism (Lohman et al., 2003; Fischer et al., 2007, 2004; Firman and Szczelkun, 2000; McClelland et al., 2005; Ali and Lohman, 1997; Fischer and Lohman, 2004; Lucius et al., 2002, 2003, 2004, 2010; Rajendar and Lucius, 2010). A generic scheme for a sequential uniform *n-step* translocation mechanism is shown in Fig. 1. According to Fig. 1, a single process occurring during the translocation of the motor protein along the filament is rate-limiting for each repeating cycle of nucleotide binding, nucleotide hydrolysis, forward motion, *etc*. The microscopic rate constant associated with this rate-limiting process is denoted as k_t in Fig. 1. Upon reaching the end of the filament, the motor protein dissociates with a microscopic rate constant k_0. The microscopic rate of dissociation from other positions along the filament is k_d. The

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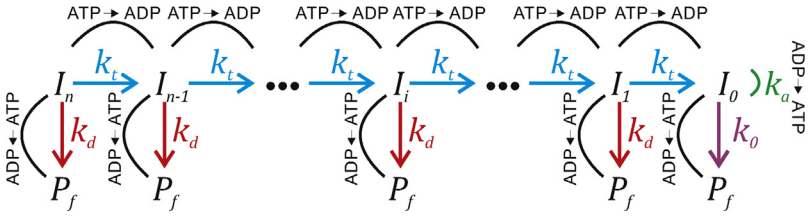

Fig. 1. Generic scheme for translocation in the sequential uniform *n-step* model. The concentration at position i is I_i, where i is defined as the number of rate-limiting translocation steps from the end of the filament. The constraint on i is that $0 \le i \le n$ where n is the maximum number of steps from one end of the filament to the other end. The microscopic rate constant associated with the rate-limiting process in the translocation reaction is k_t. Dissociation from the filament occurs at a rate of k_d except at the end where dissociation occurs at a rate of k_0. Upon dissociation, the free motor proteins (P_f) bind to a protein trap which prevents rebinding to the filament. The microscopic rates k_t, k_d, and k_0 are each associated with the binding and hydrolysis of a single ATP molecule. In addition, there is the potential for futile hydrolysis at state $i = 0$. This occurs at a rate of k_a and is not coupling to any movement of the protein.

periodicity of k_t also defines a useful coordinate system for the position of the motor protein along the filament. Specifically, each position along the filament is denoted by the number of occurrences of k_t (*i.e.*, the number of rate-limiting translocation steps) separating it from the end of the filament. The concentration of the motor protein at translocation state i is denoted by I_i.

The kinetic step-size of the motor protein denotes the distance (basepairs or nucleotides of DNA, amino acids of a polypeptide, *etc.*) moved by the motor protein along the filament between each occurrence of the rate-limiting process associated with k_t; in this manuscript we will denote the kinetic step-size by the variable m. In other words, m is the distance between each state i in Fig. 1. The product mk_t is referred to as the macroscopic translocation rate and denotes the speed of the motor protein (basepairs or nucleotides of DNA, amino acids of a polypeptide, *etc.*) per unit time. Finally the processivity of translocation – defined either microscopically or macroscopically – is a measure of the probability that the motor will translocate further along the filament rather than dissociate from the filament.

Sequential uniform *n-step* mechanisms have been used in the analysis of experimental data monitoring motor translocation to provide estimates of the associated microscopic and macroscopic kinetic parameters (Lohman et al., 2003; Fischer et al., 2007, 2004; Firman and Szczelkun, 2000; McClelland et al., 2005; Ali and Lohman, 1997; Fischer and Lohman, 2004; Lucius et al., 2004, 2003, 2002, 2010; Rajendar and Lucius, 2010). It is important to note, however, that such analysis (*i.e.*, Fig. 1) assumes that non-uniform motion (*e.g.*, backward motion, random pausing, *etc.*) does not occur (Fischer et al., 2007, 2004; Fischer and Lohman, 2004; Lucius et al., 2003; Tomko et al., 2007). This assumption is problematic, however, as the results of some experiments suggest non-uniform motion of motor proteins during translocation (Ali and Lohman, 1997; Dessinges et al., 2004; Tomko et al., 2007; Neuman et al., 2003; Perkins et al., 2004). It is therefore important to consider how the fitted kinetic parameters obtained from analysis with a sequential uniform *n-step* model are impacted when the motor protein exhibits non-uniform motion and whether the results of this analysis can provide an indication that non-uniform motion occurs; often, it is not known *a priori* whether non-uniform motion occurs.

Toward this goal, we used Monte Carlo methods to simulate the translocation of a motor protein along a filament when different non-uniform motion (*e.g.* backward motion, random pausing, jumping, and heterogeneities in the step size and the translocation rate) occurs. We then analyzed these data using a standard sequential uniform *n-step* model to determine how the presence of these non-uniform motions affect the estimates of kinetic parameters.

Our results indicate that estimates for both the macroscopic translocation rate and the macroscopic ATP coupling stoichiometry are reliably obtained using the sequential uniform *n-step* model, as determined by considering the variance between the simulated data and the model (see Tables C.3 and C.4). However, the microscopic parameters (*e.g.*, the kinetic step-size m) can be over- or under-estimated. Significantly, our results also indicate in some cases it may be possible to predict that non-uniform motion occurs based on the kinetic parameters determined using the sequential uniform *n-step* model.

2. Sequential uniform *n-step* model for translocation

2.1. Kinetic motion model

The motor protein is assumed to be bound to the filament with a contact size d (in units of nucleotides or basepairs for DNA, amino acids for polypeptides, *etc.*); the total length of the filament is denoted by L. The motor protein binds to the filament at position i which is located i rate-limiting translocation steps away from the end of the filament which is position $i = 0$ (Fig. 1). A single rate-limiting translocation step (from state i to state $i - 1$) moves the motor protein a distance of m along the filament. A maximum of n rate-limiting translocation steps is needed for a motor protein initially bound at one end of the filament to reach the other end. This means that i is constrained such that $0 \le i \le n$. The relationship between the length of the filament L, the maximum number of rate-limiting translocation steps n, the contact size d, and the kinetic step-size m can be expressed as

$$L = \mathrm{mn} + d \tag{1}$$

or

$$n = \frac{L}{m} - \frac{d}{m} \tag{2}$$

The microscopic processivity of the translocation is defined as $P \equiv k_t/(k_d + k_t)$ and is the probability of continuing to move along the filament rather than dissociating from it.

As shown in Fig. 1, each rate-limiting translocation step is associated with the binding and hydrolysis of ATP (or similar nucleotide triphosphate). Assuming c molecules of ATP are hydrolyzed for each rate limiting step (which moves the protein from position i to $i - 1$ over a physical distance m), the macroscopic ATP coupling stoichiometry is c/m. In other words, c/m is the number of ATP molecules hydrolyzed per distance translocated. Once the motor protein reaches position $i = 0$, it will dissociate from the filament at a rate of k_0. While the motor protein is at the end ($i = 0$), ATP hydrolysis that does not result in motion of the motor protein may occur. This is referred to as futile hydrolysis and occurs at the rate of k_a (Fig. 1) (Dillingham et al., 2000; Tomko et al., 2007). Inclusion of a molecular trap prevents any free motor protein that dissociates from rebinding to the filament (Fischer et al., 2004; Fischer and Lohman, 2004; Tomko et al., 2007).

2.2. Time dependence of protein concentration and ATP hydrolysis

The time dependence of the motor protein concentration I_i located at position i for the sequential uniform *n-step* model in Fig. 1 is shown in Eqs. (3)–(5) as coupled differential equations (Fischer and Lohman, 2004; Lucius et al., 2003). Note that once dissociation occurs, the motor protein is assumed to be unable to rebind to the filament.

$$\frac{d}{\mathrm{d}t} I_n(t) = -(k_t + k_d) I_n(t) \tag{3}$$

$$\frac{d}{\mathrm{d}t} I_{1 \le i < n}(t) = -(k_t + k_d) I_i(t) + k_t I_{i+1}(t) \tag{4}$$