Theory of dynamic force spectroscopy for kinetochore-microtubule attachments: rupture force distribution

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Application of pulling force, under force-clamp conditions, to kinetochore-microtubule attachments in-vitro revealed a catch-bond-like behavior. In an earlier paper (Sharma et al. Phys. Biol. (2014) the physical origin of this apparently counter-intuitive phenomenon was traced to the nature of the force-dependence of the (de-)polymerization kinetics of the microtubules. In this brief communication that work is extended to situations where the external forced is ramped up till the attachment gets ruptured. In spite of the fundamental differences in the underlying mechanisms, the trend of variation of the rupture force distribution observed in our model kinetochore-microtubule attachment with the increasing loading rate is qualitatively similar to that displayed by the catch bonds formed in some other ligand-receptor systems. Our theoretical predictions can be tested experimentally by a straightforward modification of the protocol for controlling the force in the optical trap set up that was used in the original experiments under force-clamp conditions.

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I. INTRODUCTION

Mitosis [1], the process of segregation of replicated chromosomes, is carried out in eukaryotic cells by a self-organized macromolecular machine called mitotic spindle [2–4]. One of the major components of a spindle is a stiff filament called microtubule (MT) [5] each of which has a tubular structure. The unique feature of the kinetics of a MT is dynamic instability [6]. A polymerizing MT keeps growing in length till it suffers “catastrophe” whereby it abruptly begins to depolymerize. A depolymerizing MT would, eventually, disappear unless its rapid shrinkage is stopped by a process called ‘rescue’ following which it resumes polymerization. Because of the sequence of catastrophe and rescue, a single MT can exhibit several alternate phases of polymerization and depolymerization.

On the surface of each sister chromatid, that results from DNA replication, a proteinous complex called kinetochore (kt) is located [7]. During the self-assembling of the spindle each kt attaches with one or more MTs; the actual number varies from one species to another. As the key force generators in mitosis, the MTs play crucial roles in proper positioning of the chromosomes. Equally important is the opposing force exerted by the depolymerizing MTs attached to the two sister chromatids that eventually pull the two sister chromatids apart and away from each other in the late stages of mitosis [4].

The kt-MT attachment in budding yeast is known to be the simplest; each kt can attach with only a single MT [8]. Significant progress has been made in the last few years in understanding this simple kt-MT attachment after successful reconstitution of its kinetochore in-vitro. However, the identity of all the molecular components of the coupler and its structure as well as its energetic stability and kinetics are currently under intense investigation [9–11].

One of the counter-intuitive results has been obtained from in-vitro experiments where an external force was applied on a single kt-MT attachments with an optical trap [12]. The stability was found to increase with increasing force provided the force was not too large; beyond a moderate level further increase of the force, of course, reduced the stability of the attachment. More specifically, in these “force-clamp” experiments the magnitude of the pulling force was kept fixed and the lifetime of the attachment (i.e., the time taken to get ruptured) was measured. The non-monotonic variation of the average lifetime with increasing strength of the pulling force is reminiscent of catch-bonds formed by wide varieties of ligands with their respective receptors [13–16].

In an earlier paper one of the authors (DC) of this paper (with two other co-authors) [17] developed a minimal theoretical model (from now onwards referred to as SSC model) to account for the observed catch-bond like behavior of the kt-MT attachment in budding yeast. This work elucidated the crucial role of MT kinetics (particularly its force-dependence) that makes this catch-bond fundamentally different from the common catch-bonds in chemical ligand-receptor systems in spite of their superficial similarities. It also indicated the conditions under which the same system would exhibit a slip-bond-like, instead of catch-bond like, behavior. However, the theory was developed in ref. [17] only for the force-clamp situation, i.e., for the calculation of lifetime distribution when the applied force was held constant. In this brief communication we adapt the SSC model to calculate the distribution of the rupture force under force-ramp conditions where the pulling force is increased with the passage of time. We draw attention to the similar trends of variation of the rupture force distribution reported earlier for catch-bonds formed in other ligand-receptor systems. In principle, our theoretical predictions reported here can be tested in-vitro by standard techniques of dynamic force spectroscopy [18]; a typical set up would use an optical trap with controlled ramp protocol [10].

II. SSC MODEL: FROM FORCE-CLAMP TO FORCE-RAMP

In this section we present a brief summary of the adaptation of the continuum formulation of the SSC model, as well as its discretization, that is appropriate for theoretical analysis of the force-ramp scenario. The SSC model [17] is a minimal model in the sense that it does not make any assumption about the molecular constituents or structure of the kt-MT attachment; it merely assumes a cylindrical “sleeve-like” coupler (in the spirit of the Hill sleeve model [19]) that is coaxial with the MT and has a diameter slightly larger than that of the MT. The sleeve may be an abstract representation of the Dam1 ring [20] while the “rigid rod”, that connects the sleeve with the kinetochoore, captures the effects of Ndc80 proteins [21–23].

In this model the instantaneous overlap between the outer surface of the MT and the inner surface of the coaxial cylindrical sleeve is represented by a continuous variable \( y(t) \) which is a function of time \( t \). The total length of the coupler is \( L \) so that \( 0 \leq y(t) \leq L \). Two main postulates of this model are as follows [17]:

Postulate (a): increasing overlap \( y \) lowers the energy of the system and that this lowering of energy is proportional to \( y \) so that the kt-MT interaction potential \( V_y(y) \) is assumed to have the form \( V_y(y) = -By \) where \( B \) is the constant of proportionality. Accordingly, the magnitude of the depth of the potential at \( y = L \) is \( BL \).

Postulate (b): the external force \( F \) suppresses the rate of depolymerization \( \beta \) of the MT and that \( \beta \) decreases exponentially with increasing \( F \) following \( \beta(F) = \beta_0 \exp(-F/F_c) \) where \( \beta_0 \) is depolymerization rate in the absence of any external force and the parameter \( F_c \) is a characteristic force that determines the sharpness of the decrease of
The postulate (a) is essentially a limiting case of the Hill model in the sense that the “roughness” of the interface between the outer surface of the MT and inner surface of the sleeve is neglected in the minimal version of the SSC model. The postulate (b) is qualitatively supported by the *in-vitro* experiments of Franck et al. The decrease of the rate $\beta$ with the external force $F$ need not be exponential; all the conclusions drawn from the SSC model in ref.[17] remain valid as long as the decrease of $\beta$ with increasing $F$ is sufficiently sharp.

The kinetics of this model kt-MT attachment is formulated in terms of a Fokker-Planck (FP) equation for the probability density $P(y,t)$. The attachment survives as long as $y$ remains non-zero; the rupture of the attachment is identified with the attainment of the value $y = 0$ for the first time. For the calculation of the lifetime of the attachment a unique initial condition is required. In ref.[17] the authors assumed that initially (i.e., at time $t = 0$) the MT is fully inserted into the sleeve, i.e., $y(t = 0) = L$. Since the MT is not allowed to penetrate the kinetochore plate, the overlap $y$ cannot exceed $L$. This physical condition is captured mathematically by imposing the reflecting boundary condition

$$J(y,t)|_{y=L} = 0.$$  \hspace{1cm} (1)

An absorbing boundary condition

$$P(y,t)|_{y=0} = 0$$  \hspace{1cm} (2)

is imposed at $y = 0$ for the calculation of the life times. Starting from this initial condition, the time taken by the system to attain vanishing overlap ($y = 0$ for the first time) was identified as the life time of the attachment. This lifetime fluctuates from one kt-MT attachment to another; the distribution of the lifetime contains all the statistical information.

The FP equation for $y(t)$ can be viewed as that for the position a hypothetical Brownian particle, subjected to an external potential $V(y) = -By + Fy$, in a one dimensional space with a reflecting boundary at $y = L$ and an absorbing boundary at $y = 0$. The calculation of the lifetime is essentially that of a first passage time for the Brownian particle: the time it takes to reach $y = 0$ for the first time starting from $y = L$ at $t = 0$. In ref.[17] the authors calculated the exact distribution of the lifetimes analytically in the Laplace space and hence the mean lifetime $< t >$ to be

$$< t > = \frac{D}{v^2(F)}(e^{(\frac{v(F)}{v})L} - 1) - \frac{L}{v(F)}$$  \hspace{1cm} (3)

where $D$ is the diffusion constant of the hypothetical Brownian particle while its net drift velocity $v(F)$ is given by

$$v(F) = \frac{B - F}{\Gamma} + (\alpha - \beta(F))\ell$$  \hspace{1cm} (4)

where $\ell$ is the length increased by the addition of each subunit of the MT and $\Gamma$ is the phenomenological coefficient that characterizes the viscous drag.

For the convenience of numerical computation of the distribution of the lifetimes by computer simulation, the SSC model was discretized in ref.[17] following prescriptions proposed earlier by Wang, Peskin and Elston (WPE). Excellent agreement between the results derived from the analytical theory and computer simulations was reported in ref.[17].

The external pulling force $F$ has two opposite effects on the MT. On the one hand, the MT is bodily pulled out of the coupler by $F$. On the other hand, because of the suppression of the depolymerization rate $\beta$ by the external pull $F$, the polymerization can dominate over depolymerization resulting in a net growth of the MT. If the increase in $y$ resulting from the net growth of the MT can more than compensate the decrease in $y$ caused by the bodily movement of the MT out of the coupler, the net result will be an increase of $y$. Such an increase of $y$, instead of the naively expected decrease, upon application of $F$ would be interpreted as an effective increase of the stability of the kt-MT attachment with increasing strength of the applied force $F$. However, as the strength of $F$ increases, $\beta(F)$ gradually saturates. Since $\beta$ practically stops decreasing further with the further increase of $F$ the bodily movement of the MT out of the coupler at higher values of $F$ can no longer be compensated by the tip growth into the coupler; the net decrease of $y$ with further increase of $F$ in this regime manifests as decrease in the stability of the kt-MT attachment. Such overall non-monotonic variation of $< t >$ with $F$ is interpreted as a catch bond-like behavior of the kt-MT attachment. However, increase of $< t >$ with $F$ in the small $F$ regime is possible only if $\beta(F)$ decreases sufficiently sharply with increasing $F$. Otherwise, the kt-MT attachment would behave effectively as a slip bond.

In ref.[17] the external $F$ was assumed to be independent of time $t$; these corresponds to a “force-clamp” situation in the experiments. In contrast, in this paper the time-dependent external force $F(t)$ is assumed to increase according
FIG. 1. (a) Linearly increasing force \( F = at \); different straight lines correspond to different rates of loading. The \( k_t \)-MT attachment survives the increasing tension up to a certain time and then gets ruptured. (b) A schematic depiction of the \( k_t \)-MT attachment in the presence of external force. (c) Hypothesized effective potentials \( V_b(y) \) and \( V_f(y,t) \) are plotted against the instantaneous length of overlap \( y(t) \).

to a well defined protocol; this corresponds to a “force-ramp” in experiments. We adopt the postulates (a) and (b) of the SSC model. For the sake of simplicity, we assume a linear ramp force, namely, \( F(t) = at \) where \( a \) is loading rate. The instantaneous external force \( F(t) \) can be derived from the corresponding instantaneous potential landscape, \( V_f(y,t) = F(t)y \). The effective potentials \( V_b(y) \) and \( V_f(y,t) \) at an arbitrary instant of time are plotted in fig.1. Net instantaneous potential \( V(y,t) \) felt by the kinetochore is \( V(y,t) = V_b(y) + V_f(y,t) \).

For the theoretical treatment of the \( k_t \)-MT attachment subjected to a ramp force \( F(t) \), we adapt the corresponding theory for ligand-receptor bond rupture, developed originally by Bell [28] and subsequently extended by Evans and Ritchie [29] and by Evans and Williams [30]. In the presence of a given force \( F \), let \( k_{on}(F) \) and \( k_{off}(F) \) be the rates of binding and unbinding, respectively, of a MT to the \( k_t \) mediated by the coupler. Because of the specific choice of the initial condition \( y(t = 0) = L \) and the absorbing boundary condition at \( y = 0 \), no rebinding of the MT is possible and, therefore, we can put \( k_{on}(F) = 0 \). Denoting the probability that \( y \neq 0 \) (i.e., MT is attached to the \( k_t \)) at time \( t \) by the symbol \( P_{on}(t) \), the equation governing the time evolution of \( P_{on}(t) \) is

\[
\frac{dP_{on}(t)}{dt} = -k_{off}(F)P_{on}(t).
\]  
(5)

Hence, in terms of \( k_{off}(F) \), the survival probability \( S(t) \) of the attachment (i.e., the probability that the hypothetical Brownian particle has not reached \( y = 0 \) before time \( t \)) can be expressed as [31]

\[
S(t) = \exp \left[ -\int_0^t k_{off}(F(t'))dt' \right]
\]  
(6)
Moreover, in terms of $k_{off}(F)$ the probability density $\rho_{fp}(F)$ of the rupture forces is expressed as [31]

$$\rho_{fp}(F) = \frac{k_{off}(F)}{a} \left[ \exp \left( -\frac{1}{a} \int_0^F k_{off}(F')dF' \right) \right]$$

(7)

Thus, for the calculation of $S(t)$ and $\rho_{fp}(F)$ the analytical expression for $k_{off}(F)$ is required. For $k_{off}(F)$ we use the expression for the inverse of the average lifetime of a single kt-MT attachment in the SSC model, reported in ref.[17], namely,

$$k_{off}(F) = \frac{1}{<t>} = \frac{v^2(F)}{D\left(e^{\frac{aF}{L}} - 1\right) - Le(F)}$$

(8)

where the expression $v(F)$ is given by Eq.(4). Substituting Eq.(5) into the Eqs.(4) and (7) we get, respectively, the survival probability $S(t)$ and the rupture force density $\rho_{fp}(F)$ by numerically evaluating the respective integrals.

For computer simulation of the model, we discretize the continuum version of the SSC model following WPE prescription [26, 27] as explained in ref.[17]. Instead of a constant force, a time-dependent external force $F = at$ is imposed. carrying out computer simulations of this discretized version of the model we directly compute the survival probability $S(t)$ and the distribution $\rho_{fp}(F)$ of the rupture forces. Throughout this paper lines and discrete points, respectively, have been used to plot the theoretical results derived from numerical integrations of eqns. (6)-(7) and the data obtained from computer simulations of the discretized model. Parameter values that we used in simulation are listed in Table I.

### III. RESULT FOR THE RUPTURE OF KT-MT ATTACHMENT UNDER RAMP FORCE

In the Fig.2(a) the rupture force distribution obtained from numerical integration of the eqns. (6)- (7) of the continuum theory and those obtained from computer simulation of the discretized model are plotted for four different loading rates. At loading rates as low as $a = 3 \times 10^{-3} pN s^{-1}$ (violet), the most probable rupture force is vanishingly small. At such slow loading rates the rupture of the attachment is mostly spontaneous dissociation caused by thermal fluctuation and is very rarely driven by the applied tension. However, as the loading rate increases a second peak at a non-zero value of the force begins to emerge. At moderate loading rates like $a = 1 \times 10^{-3} pN s^{-1}$ (blue line and triangle ) and $a = 3 \times 10^{-3} pN s^{-1}$ (green line and square)), a large fraction of the kt-MT attachments survive up to a high force before getting ruptured while another significant fraction of the attachments still dissociate at a vanishingly small force. But, at sufficiently high rates of loading, for example at $a = 3 \times 10^{-2} pN s^{-1}$ (red), an overwhelmingly large fraction survives up to a high force while very few attachment get ruptured by very weak forces. The existence of two peaks at intermediate rates of loading, where the peak at non-zero force rises with increasing loading rate while that at the vanishing force decreases, is a key signature of catch bonds established by force-ramp experiments with other well known catch bonds [?].

In the Fig.2(b) the survival probabilities are plotted at the same loading rates for which the rupture force distributions have been plotted in Fig.2(a). For the same set of parameter values, the data in the Figs 2(a) and (b) are consistent with each other. At very high loading rates the probability of survival remains high, and practically unaffected by the applied force, up to quite high values of the force and, accordingly, the most probable rupture force is also expected to be high. In contrast, sharp drop in the survival probability with increasing force is also reflected in the vanishingly small most probable rupture force at very low loading rates. In the Fig.2(c) we have plotted mean rupture force as a function of loading rate. Mean rupture force increases with increasing loading rate.

| Parameter                                         | Values                        |
|---------------------------------------------------|-------------------------------|
| Inter-space between MT binding site $l$ [19,32,33] | 8/13 nm                       |
| Total length of coupler $L$                       | 50 nm                         |
| Polymization rate $\alpha$ [19,32,33,38]         | 20-50 s$^{-1}$                 |
| Maximum Depolymization rate $\beta_0$ [19,32,33,38]| 100-350 s$^{-1}$              |
| Characteristic force of Depolymization $F_\beta$ [17] | 0.8 pN                        |
| Attractive force between kt-MT $B$ [17]           | 1.9 pN                        |
| Diffusion constant $D$ [19,32,33]                 | 700 nm$^2$s$^{-1}$            |
| Viscous drag coefficient $\Gamma$ [19,32,33,39]  | $6pN\mu\text{s}^{-1}$         |

**TABLE I.** Values of the parameters for kt-MT system
The increase of mean and most probable rupture force with increasing loading rate is also observed in case of common ligand-receptor attachments [31]; it follows from the mathematical form of the equation

$$\frac{dP_{\text{on}}(F)}{dF} = -\frac{1}{a}k_{\text{off}}(F)P_{\text{on}}(F)$$  

(9)

which is nothing but the equation (5) expressed in terms of force $F$ rather than time $t$. Eqn. (9) implies that the rate of decay of the bound state of the bond is inversely proportional to the loading rate $a$. Consequently, the ligand-receptor bond persists up to higher values of force when subjected to faster loading rates.
IV. SUMMARY AND CONCLUSION

The strength and stability of non-covalent ligand-receptor bonds are routinely probed by dynamic force spectroscopy \cite{[18]} . The rupture of such weak bonds is a thermally activated process; the external force alters the potential landscape thereby affecting its strength and stability, i.e., assist or oppose the thermally activated rupture process. Because of the intrinsic stochasticity of the rupture process the rupture force itself is a randomly distributed quantity. Therefore, repetition of the force-ramp experiment yields the probability density of the rupture force.

In this brief communication we have adapted the SSC model of rupture of kt-MT attachment in force-clamp set up to force-ramp scenario. Experimental data corresponding to the force-clamp set up have been reported \cite{[12]} . Theories at different levels of molecular detail have been developed in the last few years to account for some unusual trends of variation observed with varying force in those experimental data \cite{[12, 17, 40]} . In this paper we have discovered signatures of the catch-bond-like behaviors of the kt-MT attachments in the force-ramp studies within the framework of the SSC model.

In the force-clamp set up with optical trap, the bead-trap separation is maintained at a fixed value with a computer controlled feedback while the change in the length of the MT is recorded by monitoring the movement of the specimen stage \cite{[10]} . A force-ramp set up, where the bead-trap separation is gradually increased with time, has also been designed by modifying the force-clamp software \cite{[10]} . This force-ramp can be used to test the theoretical predictions made in this brief communication.

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