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A model for dynamics of primer extension by eukaryotic DNA primase

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Abstract A mathematical model is proposed for processive primer extension by eukaryotic DNA primase. The model uses available experimental data to predict rate constants for the dynamic behavior of primase activity as a function of NTP concentration. The model also predicts some data such as the binding affinities of the primase for the DNA template and for the RNA primer.

Keywords DNA primase · Primer synthesis · Model

Introduction

Even though DNA polymerases duplicate all of the DNA during DNA replication, they require an RNA polymerase to initiate chain synthesis. Primase is the special RNA polymerase that synthesizes a short RNA primer from which the DNA polymerase elongates. Most primases initiate synthesis complementary to specific template sequences and then synthesize RNA polymers that can be anywhere from 3 to 30 nucleotides long (Frick and Richardson 2001; Arezi and Kuchta 2000; Corn and Berger 2006; Hamdan and Richardson 2009; Kuchta and Stengel 2010).

In eukaryotes, the initiation of new DNA strands is performed by DNA polymerase α (pol α)–primase complex, co-purifying as a four-peptide complex, with subunits of approximately 49, 58, 70, and 180 kDa. The p49 and p58 subunits constitute the primase, the p180 subunit contains pol α catalytic activity, while the p70 subunit may be important for the p180 production and nuclear localization as well as tethering the pol α-primase complex to the replication fork via interactions with other proteins found at the fork (Arezi and Kuchta 2000; Kaguni et al. 1983; Goulian and Heard 1989; Cotterill et al. 1987; Lehman and Kaguni 1989). To initiate the template-directed synthesis of an RNA primer, the primase firstly binds two NTPs in addition to the ssDNA template, forming a primase-ssDNA-NTP–NTP quaternary complex. The complex undergoes catalysis to generate the dinucleotide RNA primer. The primase then catalyzes the processive extension of the dinucleotide, ultimately generating RNA primer of approximately 7–10 nucleotides in length (unit-length primer) (Sheaff and Kuchta 1993). The unit-length primer is then transferred intra-molecularly to the pol α and then is elongated by the pol α via polymerization of about 20 dNTP (Sheaff et al. 1994; Arezi and Kuchta 2000).

In this work, on the basis of the available experimental evidence, a model is proposed for the processive synthesis of the RNA primer by the eukaryotic DNA primase. Based on the model, the dynamic behavior of primase activity as a function of NTP concentration is studied, with the theoretical results being in good quantitative agreement with the experimental data. The model also predicts some data for the primase such as the NTP-binding rate and the binding affinities for the DNA template and for the RNA primer.

Model for processive primer extension by primase

Several key findings were used to develop the model for processive synthesis. It has been shown that the DNA-binding and RNA-binding sites in the p49–p58 primase complex are located in different subunits. Specifically, the DNA-binding site is located in the p58 subunit since it is

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capable of binding DNA before and after primer synthesis (Arezi et al. 1999). Purified recombinant p49 subunit has enzymatic activity and binds RNA, whereas purified p58 does not (Schneider et al. 1998). Thus, the p58 subunit binds primarily to the DNA strand, while the p49 subunit binds primarily to the RNA strand but also maintains some contact with the DNA strand. For simplicity in the treatment it is assumed that the p49 subunit only binds the RNA strand and the binding site can cover m RNA bases. To develop the model for counting primer length, it was assumed that the linking residues between the two subunits (p49 and p58) behave elastically until the maximum length is reached.

Model

Based on the above, the model for processive primer extension by the primase is presented in this section. In the description of the model, we take that the RNA-binding site in the p49 subunit can bind $m = 7$ RNA bases. As we will show in Results, m should be larger than or equal to 6 in our model.

Firstly, the p58 subunit binds ssDNA, where the p49 subunit is located at the equilibrium position relative to the p58 subunit. Due to the thermal noise, the primase can slide randomly along the ssDNA template (Sheaff and Kuchta 1993). After two NTPs bind, which enhances the affinity between the p58 subunit and the DNA, the p58 subunit becomes bound strongly to the DNA (Kirk et al. 1997). Then, the two NTPs are incorporated slowly (Sheaff and Kuchta 1993), generating a dinucleotide RNA primer (Fig. 1a).

The ensuing binding of the RNA-binding site in the p49 subunit to the dinucleotide RNA primer repositions the p49 subunit along the DNA template, inducing the p49 subunit to translocate forward along the DNA template and making the active site be located at the position opposite to the next unpaired base (i.e., the third base) on the template (Fig. 1b). Note that the translocation-induced deviation of the p49 subunit from its equilibrium position relative to the p58 subunit, which is binding to the DNA template at the fixed site, results in the occurrence of an internal elastic force acting on the two subunits. Then an NTP complementary to the sterically corresponding unpaired base (i.e., the third base) on the template binds to the active site. The incorporation of the NTP generates the trinucleotide RNA primer. Since the affinity of the RNA-binding site for the RNA primer of three bases is larger than that of two bases, the p49 subunit would translocate one base so that the RNA-binding site binds three bases of the RNA primer (Fig. 1c). The

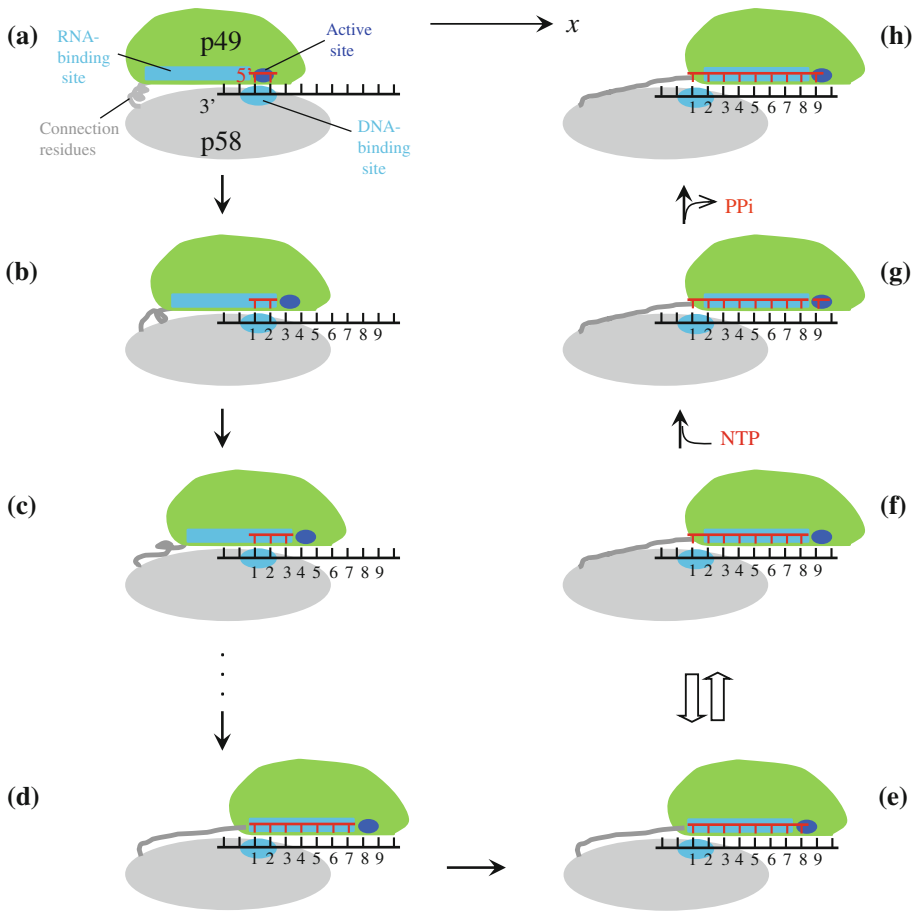

Fig. 1 Schematic illustrations of the model for processive extension of primer by primase (see text for detailed description). For clarity, the DNA template is drawn in *black*, while the generated RNA primer in *red*

translocation makes the active site be located at the position opposite to the fourth base on the template. Note that the translocation also results in the increase of the internal elastic force. Then the fourth NTP binds and is incorporated. Similarly, the fifth, sixth and seventh NTPs are successively incorporated. After the incorporation of the seventh NTP, the active site of the p49 subunit becomes located at the position opposite to the eighth unpaired base on the template and the RNA-binding site now becomes bound to all m RNA bases that the binding site can bind (Fig. 1d). It is noted that, in the above description, it is implicitly considered that the restriction of the structure of the p49 subunit complexed with the primer-template does not allow the primer 3′-terminus to move forwards relative to the p49 subunit when its active site is located at the 3′-terminus, as it is seen from the structure of other nucleic-acid polymerases such as DNA polymerase Dpo4 (Wong et al. 2008).

After the eighth NTP binds and is then incorporated, whether the p49 subunit translocates forward one base or not, the RNA-binding site has the same affinity for the primer, because for both cases the RNA-binding site binds $m = 7$ RNA bases (see Fig. 1e, f). Now, the thermal noise can induce the active site to jump between the position located at the 3′-terminus (opposite to the eighth base on the template) (Fig. 1e) and the position opposite to the ninth unpaired base (Fig. 1f). The occupancy of the active site by the primer 3′-terminus (Fig. 1e) sterically prevents an NTP from binding to the active site. However, during the period when the active site is located at the position opposite to the ninth unpaired base (Fig. 1f), since the active site is nucleotide free, an NTP becomes able to bind to it. The occupation of the active site by the NTP sterically prevents the p49 subunit from moving backwards to the position opposite to the eighth base, thus stabilizing the active site at the position opposite to the ninth unpaired base (Fig. 1g). Then the bound NTP is incorporated (Fig. 1h). It is noted here that, as the deviation distance of the p49 subunit from the p58 subunit increases more and more, the residues that connect the two subunits are stretched longer and longer. When the connection is stretched to its maximum length, the p49 subunit cannot move to the next position. This stalling explains how primase has the ability to "count."

Results

The absence of DNA-binding site in primase inhibits primer initiation, decreases primase processivity and loses primase counting ability

Based on the model, if there is no affinity of the mutant p58 subunit for DNA, it is evident that the primer initiation, i.e., the dinucleotide synthesis, cannot proceed. If the mutant p58 subunit has a weaker affinity for DNA than the wild type, the processivity of primer extension would be decreased due to the lower binding affinity of the mutant p58–p49 complex for the primer-template than the wild-type p49–p58 complex. Moreover, in the present model, the counting ability of the primase is due to the limited distance of the p49 subunit that can be deviated from the p58 subunit when the p58 subunit is bound strongly to DNA. Thus, without the interaction of the p58 subunit with the DNA, the counting ability of the primase (e.g., either the mutant p58–p49 complex or the p58 subunit alone) would be lost although the primer-extension ability remains. All these deductions are in agreement with the experimental results, showing that mutations to the p58 subunit inhibit primer initiation, decrease primase processivity, and lose primase counting ability (Zerbe and Kuchta 2002).

Dissociation dynamics of p49 subunit from RNA primer during primer extension

During primer extension, the movement of the p49 subunit along the direction perpendicular to the DNA template can be described by the following Langevin equation

$$\Gamma \frac{\mathrm{d}y}{\mathrm{d}t} = -\frac{\mathrm{d}\left[U_0 W_{p49}(y)\right]}{\mathrm{d}y} + \xi(t), \tag{1}$$

where y represents the position of the p49 subunit along the direction perpendicular to the DNA template. For simplicity, we approximate the p49 subunit as a sphere. Since it seems impossible to derive a formula for the drag coefficient on the sphere when a polymer chain, i.e., the DNA template, on one side of the sphere is present, we assume here that the drag coefficient on the p49 subunit can be estimated roughly with Stokes formula, $\Gamma = 6\pi\eta R$, as used elsewhere to describe a motor protein moving near its track (Mogilner et al. 1998, Fox and Choi 2001; Xie 2008, 2009), where $\eta = 0.01$ g cm^{-1} s^{-1} is the viscosity of the solution and $R = 3$ nm is the radius of the p49 subunit. $U_0 W_{p49}(y)$ is the interaction potential of the RNA-binding site in the p49 subunit with RNA primer along the y direction, where U_0 is a constant dependent of the number of RNA bases generated and $W_{p49}(y)$ can be written in the Morse form, $W_{p49}(y) = \exp(-2y/d) - 2\exp(-y/d)$, with $2d = 1$ nm (the Debye length). Based on the model, $U_0 = 2E$ after the generation of the dinucleotide primer but before the incorporation of the third RNA bases, $U_0 = nE$ after the generation of the primer of n bases but before the incorporation of the $(n + 1)$th RNA bases $(2 < n < m)$, and $U_0 = mE$ after the generation of the primer of n bases $(n \geq m)$, where E is the binding affinity per base for the RNA primer and m is the number of RNA bases that the

RNA-binding site of the p49 subunit can bind. The last term in Eq. 1, $\xi(t)$, represents the thermal noise, with $\langle\xi(t)\rangle = 0$ and $\langle\xi(t)\xi(t')\rangle = 2k_BT\Gamma\delta(t-t')$.

From Eq. 1 the mean dissociation time of the p49 subunit from the RNA primer, i.e., the mean first-passage time for the p49 subunit to move along the y direction by a distance $L > 2d$, is obtained as follows (Xie 2011)

$$T_d = \frac{\Gamma}{k_BT}\int_0^L \exp\left\{\frac{U_0}{k_BT}\left[\exp\left(-\frac{2y}{d}\right) - 2\exp\left(-\frac{y}{d}\right)\right]\right\}\mathrm{d}y \times \int_0^y \exp\left\{-\frac{U_0}{k_BT}\left[\exp\left(-\frac{2z}{d}\right) - 2\exp\left(-\frac{z}{d}\right)\right]\right\}\mathrm{d}z \tag{2}$$

Using Eq. 2 the calculated results of the mean dissociation time T_d versus U_0 is shown in Fig. 2a, where we take $L = 5$ nm $> 2d = 1$ nm. It is seen that T_d increases significantly and nearly exponentially with the increase of U_0 when $U_0 > 5\ k_BT$. The significant increase of T_d with the increase of U_0 implies that, during the synthesis of the unit-length primer, as the number of the generated RNA bases increases, the dissociation probability of the p49 subunit from the newly generated RNA primer decreases significantly. After the synthesis of m RNA bases, where m is the number of RNA bases that the RNA-binding site of p49 subunit can bind, the dissociation probability is kept to be the minimum value. This also implies that, during the synthesis of the primer dimer in the absence of dNTP or pol α, the p49 subunit has the constant small probability to dissociate from the generated RNA primer.

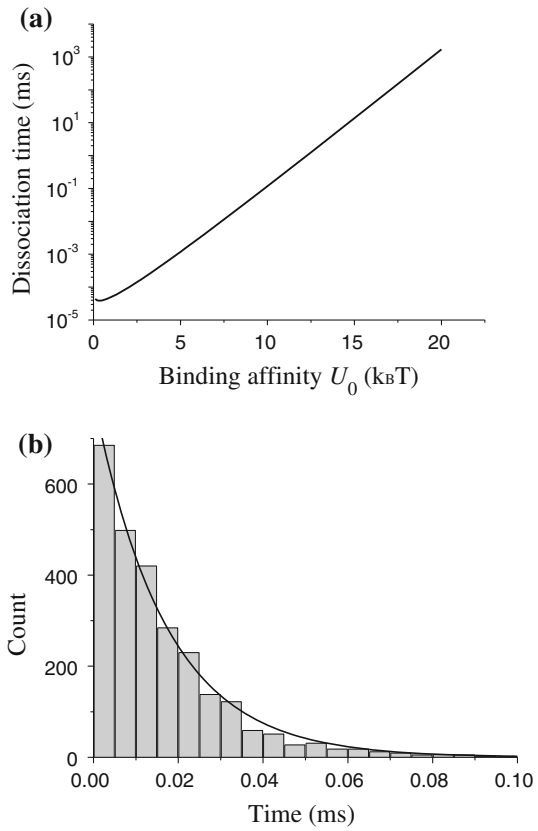

Fig. 2 Results for dissociation dynamics of the p49 subunit from the RNA primer. **a** Mean dissociation time T_d of the p49 subunit from the RNA primer versus the binding affinity U_0 between them. **b** Time distribution of the p49 subunit dissociating from the RNA primer with $U_0 = 8\ k_BT$. The *line* is the single-exponential fitting with the time constant equal to the mean dissociation time T_d

To determine the time distribution of the p49 subunit dissociating from the RNA primer, we numerically solve Eq. 1 by using the Stochastic Runge-Kutta method, as used elsewhere (Xie 2008; Xie and Sayers 2011). A result for the dissociation-time distribution with $U_0 = 8\ k_BT$ is shown in Fig. 2b. It is seen that the distribution can be well fitted to the single-exponential function, i.e., $f_d(t) = A\exp(-t/T_d)$, where A is constant.

Amount of RNA primer products versus NTP concentration

From Eq. 12 (see "Appendix 2"), the probability for the primase to synthesize a dinucleotide primer, $P^{(2)}$, after it binds to the ssDNA template can be obtained as follows

$$P^{(2)} = \frac{[\mathrm{NTP}]}{[\mathrm{NTP}] + K^{(2)}}, \tag{3}$$

where $K^{(2)} = 1/(k_b^{(2)}T_d^{(2)})$, with $k_b^{(2)}$ being the NTP-binding rate and $T_d^{(2)}$ being the mean dissociation time of the primase from the DNA template before the binding of two NTPs. Note that $P^{(2)}$ approaches 1 at saturating [NTP] ($[\mathrm{NTP}] \gg K^{(2)}$), implying that the primase has a negligible probability to dissociate from the DNA template after two NTPs bind, which is consistent with the experimental data of Kirk et al. (1997) showing that, under conditions of high NTP concentration, primer synthesis occurs at the first potential synthesis site to which primase binds (see "Appendix 2").

From Eq. 14 (see "Appendix 3"), the probability for the primase to incorporate the $(n+1)$th RNA base that is connected to the RNA primer of n bases ($n \geq 2$) reads

$$P_n = p_p^{(n)}\frac{[\mathrm{NTP}]}{[\mathrm{NTP}] + K_n}, \tag{4}$$

where $p_p^{(n)}$ denotes the probability for the primase to complete the incorporation of the $(n+1)$th RNA base after the NTP binds and $K_n = 1/\left(k_{b(n+1)}T_{dn}\right)$, with $k_{b(n+1)}$ being the NTP-binding rate for the $(n+1)$th NTP, which gives $k_{b(n+1)}[\mathrm{NTP}] = 1/T_{b(n+1)}$, and T_{dn} being the mean

dissociation time of the p49 subunit from the RNA primer of n bases. Note that, during the processive primer extension, $k_{b(n+1)}$ has nearly the same value for any $n \geq 2$. However, it is noted from Fig. 2a that T_{dn} increases nearly exponentially with the increase of n. For the value of the binding affinity $E = 4.3\ k_BT$ per RNA base, from Fig. 2a we have $T_{d(n+1)} \approx CT_{dn}$ if $n + 1 \leq m$, where $C \gg 1$ is a constant, nearly independent of $n \geq 2$. Thus, we have $K_{(n+1)} \ll K_n$, with $K_{(n+1)} \approx K_n/C$.

It is known that, after the p49 subunit dissociates from the newly generated dinucleotide primer, the affinity of the p58 subunit for the DNA is reduced and, thus, the p58 subunit then slides along the DNA template to another site to reinitiate another round of primer synthesis. Consequently, for one binding event of primase to the DNA template, there would be $1/P_2 = ([\mathrm{NTP}] + K_2)/(p_p^{(2)}[\mathrm{NTP}])$ rounds of the dinucleotide synthesis. In addition, as mentioned above, $K_{(n+1)} \ll K_n\,(n \geq 2)$. As a result, for approximation, we can neglect the contribution to the dinucleotide synthesis resulting from the small dissociation probability of the p49 subunit from the newly generated RNA primers of $n \geq 3$ bases. Thus, the total number of the generated primers that include both the abortive and unit-length primers for one binding event of the primase to the DNA template can be approximately calculated by

$$N_{\mathrm{Total}} = \sum_{i=1}^{1/P_2} \left(P^{(2)}\right)^i = \sum_{i=1}^{([\mathrm{NTP}]+K_2)/\left(p_p^{(2)}[\mathrm{NTP}]\right)} \left(\frac{[\mathrm{NTP}]}{[\mathrm{NTP}] + K^{(2)}}\right)^i, \tag{5}$$

which is finally obtained as

$$N_{\mathrm{Total}} = \frac{[\mathrm{NTP}]}{K^{(2)}}\left[1 - \left(\frac{[\mathrm{NTP}]}{[\mathrm{NTP}] + K^{(2)}}\right)^{\frac{[\mathrm{NTP}]+K_2}{\left(p_p^{(2)}[\mathrm{NTP}]\right)}}\right]. \tag{6}$$

From experimental data of Kuchta et al. (1990), it is obtained that the probability for the primase to incorporate the third RNA base after the NTP binds, $p_p^{(2)} = \left(k_p/k_d\right)/\left(1 + k_p/k_d\right) = 0.4$, where k_p is the polymerization rate and k_d is the dissociation rate of the p49 subunit from the dinucleotide primer at saturating [NTP], with $k_p/k_d = 0.67$ (Kuchta et al. 1990). Thus, in Eq. 6 there are only two unavailable parameters $K^{(2)}$ and K_2. Taking $K^{(2)} = 0.28\ \mu\mathrm{M}$ and $K_2 = 390\ \mu\mathrm{M}$, the calculated results of N_{Total} versus [NTP] are shown in Fig. 3a (line). It is seen that with only two adjustable parameters the theoretical results are in good agreement with the experimental data (Fig. 5b in Sheaff and Kuchta 1993). The small value of $K^{(2)}$ implies the long mean dissociation time $T_d^{(2)}$ of the primase from the DNA template before the binding of two NTPs, which is consistent with the experimental results (Sheaff and Kuchta 1993).

From Eq. 6, the number of unit-length primers can be approximately calculated by

$$N_{\mathrm{Unit}} = \frac{[\mathrm{NTP}]}{K^{(2)}}\left[1 - \left(\frac{[\mathrm{NTP}]}{[\mathrm{NTP}] + K^{(2)}}\right)^{\frac{[\mathrm{NTP}]+K_2}{\left(p_p^{(2)}[\mathrm{NTP}]\right)}}\right] \times \prod_{n=2}^{m} \frac{p_p^{(n)}[\mathrm{NTP}]}{[\mathrm{NTP}] + K_2/C^{n-2}}, \tag{7}$$

where C is related to the binding affinity per RNA base, E, of the p49 subunit for RNA primers through Fig. 2a and $p_p^{(n)}$ $(n = 3, \ldots, m)$ can be calculated by using experimental data of Kuchta et al. (1990), as done for $p_p^{(2)}$ in Eq. 6. In Eq. 7, the two parameters $K^{(2)} = 0.28\ \mu\mathrm{M}$ and $K_2 = 390\ \mu\mathrm{M}$, as determined in Fig. 3a, and thus there is only one unavailable parameter C. Taking $C = 60$, the calculated results of

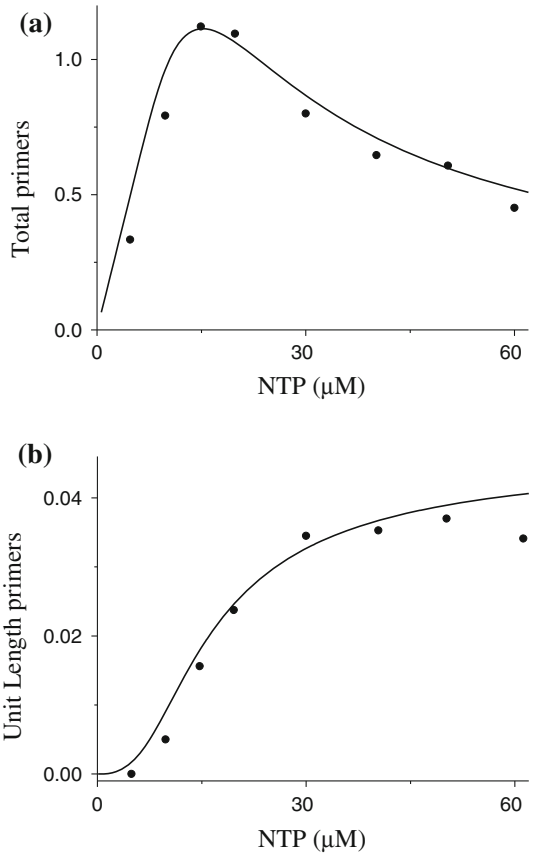

Fig. 3 Numbers of the generated primers for one binding event of primase to the DNA template versus NTP concentration, [NTP]. **a** Total number, N_{Total}, of the generated primers that include both the abortive and unit-length primers (*line*), where for comparison with the experimental data (*dots*) the calculated values of N_{Total} are divided by 34.3. **b** Number, N_{Unit}, of the generated unit-length primers (*line*), where for comparison with the experimental data (*dots*) the calculated values of N_{Unit} are divided by 12.9. The experimental data (*dots*) are taken from Sheaff and Kuchta (1993)

N_{Unit} versus [NTP] are shown in Fig. 3b (line). It is seen that the theoretical results of N_{Unit} versus [NTP] is also in agreement with the experimental data (dots) (Fig. 5a in Sheaff and Kuchta 1993).

Interestingly, from Fig. 2a, we see that $C = 60$ corresponds to the binding affinity per RNA base, $E = 4.3\ k_BT$. Thus, the binding affinity of the p49 subunit for the dinucleotide RNA primer is about $U_0 = 2E = 8.6\ k_BT$, which gives $T_{d2} = 0.032$ ms (see Fig. 2a). From $K_2 = 1/(k_{b3}T_{d2}) = 390\ \mu\text{M}$ it is obtained that $k_{b3} = k_{bn} = 80\ \mu\text{M}^{-1}\ \text{s}^{-1}$ ($n > 3$). This implies that, during the processive primer extension, the NTP-binding rate is estimated to be $80\ \mu\text{M}^{-1}\ \text{s}^{-1}$.

It is noted that, in Eqs. 6 and 7, for approximation, we have neglected the contribution to the dinucleotide synthesis resulting from the dissociation probability of the p49 subunit from the newly generated RNA primers of $n \geq 3$ bases, which is much smaller than that from the dinucleotide primers. Nevertheless, the theoretical results for both the amount of the unit-length primers and the total amount of primers that include both the abortive and unit-length primers are consistent with the experimental data. It is expected that the inclusion of the contribution resulting from the dissociations from the RNA primers of $n \geq 3$ bases would give a much better agreement with the experimental data.

The absence of dNTP or pol α results in the generation of primer dimers

Experimental results showed that the rate of unit-length primer synthesis is about $k_{\text{RNA}} = 0.003\ \text{s}^{-1}$ and the rate of product formation (i.e., the unit-length primer synthesis plus the ensuing primer elongation) by the coupled pol α–primase complex is about $k_{\alpha-p} = 0.002\ \text{s}^{-1}$ (Sheaff et al. 1994). Denoting k_{DNA} the rate of the primer elongation by pol α, we have the relation, $k_{\alpha-p} = k_{\text{RNA}}k_{\text{DNA}}/k_{\text{RNA}}k_{\text{DNA}}(k_{\text{RNA}} + k_{\text{DNA}}).(k_{\text{RNA}} + k_{\text{DNA}})$. From this relation, we obtain that, following the completion of the unit-length primer synthesis, the rate of the primer elongation is about $k_{\text{DNA}} = 0.006\ \text{s}^{-1}$. This implies that the mean time for the transfer of the unit-length primer from the primase to pol α is about $T_{\text{transfer}} = 170$ s.

To ensure the transfer of the unit-length primer from the primase to pol α, it is required that both the time for the p58 subunit to dissociate from the DNA and the time for the p49 subunit to dissociate from the unit-length RNA primer $T_d > T_{\text{transfer}}$. From Fig. 2a we see that $T_d = T_{\text{transfer}} = 170$ s corresponds to an affinity of about $24.7\ k_BT$. Thus, it is required that both the affinity of the p58 subunit for the DNA, $E_{p58} > 24.7\ k_BT$, and the affinity the p49 subunit for the unit-length primer, $E_{p49} = U_0 = mE > 24.7\ k_BT$, where m is an integer and $E = 4.3\ k_BT$, as determined in above section. Thus, we have $m \geq 6$.

Now, consider the absence of dNTP or pol α in solution. Based on the model, after the synthesis of the unit-length primer, the p49 subunit cannot move to the next unpaired base along the DNA template due to the finite maximum length of the connection residues between the two subunits. Since now the RNA-binding site of the p49 subunit binds all m RNA bases that the binding site can bind, the RNA-binding site has a high affinity U_0 for the RNA primer, with $U_0 = mE \geq 25.8\ k_BT$ for $m \geq 6$. From Fig. 2a, it is seen that the mean dissociation time of the p49 subunit from the RNA primer is $T_d \geq 477.5$ s at $U_0 \geq 25.8\ k_BT$, implying that the p49 subunit would take a very long time to dissociate from the RNA primer. Moreover, since in the absence of dNTP or pol α the primer cannot be transferred from the primase to pol α, the primase thus becomes stalled.

Consider that the p58 subunit binds to the DNA with an affinity, $E_{p58} < E_{p49} = mE \geq 25.8\ k_BT$. Then, during the stalled period of the primase, it is most probable that only the p58 subunit that contains the DNA-binding site dissociates from the DNA template. After the dissociation, the movement of the p58 subunit with respect to the p49 subunit that is bound fixedly to the RNA primer can be described by the following Langevin equation

$$\Gamma\frac{\mathrm{d}x}{\mathrm{d}t} = C_p(X_0 - x) + \xi(t), \tag{8}$$

where $\Gamma = 6\pi\eta R = 5.65 \times 10^{-11}\ \text{kg s}^{-1}$ and C_p is the elastic coefficient of the residues connecting the subunits p58 and p49. The initial and final positions of the p58 subunit are at $x = 0$ (Fig. 4a) and $x = X_0$ (Fig. 4b), respectively, where X_0 is the maximum length of the residues connecting the two subunits. Here we take $X_0 = 10p = 3.4$ nm. From Eq. 8 the mean first-passage time for the p58 subunit to move from $x = 0$ to $x = X_0$ is obtained as follows

$$T_m = \frac{\Gamma}{k_BT}\int_0^{X_0}\exp\left[\frac{1}{2k_BT}C_p(X_0 - y)^2\right]\mathrm{d}y \times \int_0^{y}\exp\left[-\frac{1}{2k_BT}C_p(X_0 - z)^2\right]\mathrm{d}z. \tag{9}$$

Using Eq. 9, the calculated results of T_m versus C_p are shown in Fig. 5. As expected, T_m decreases with the increase of C_p. It is seen that T_m is rather short: even for $C_p = 5$ pN/nm, T_m is only about 0.022 μs.

After the p58 subunit rapidly returns to the equilibrium position relative to the p49 subunit, the DNA-binding site on the p58 subunit rebinds to the DNA template, as schematically shown in Fig. 4. Then, similar to the synthesis of the unit-length primer, the primase would continue to synthesize the next unit-length primer. It is thus deduced

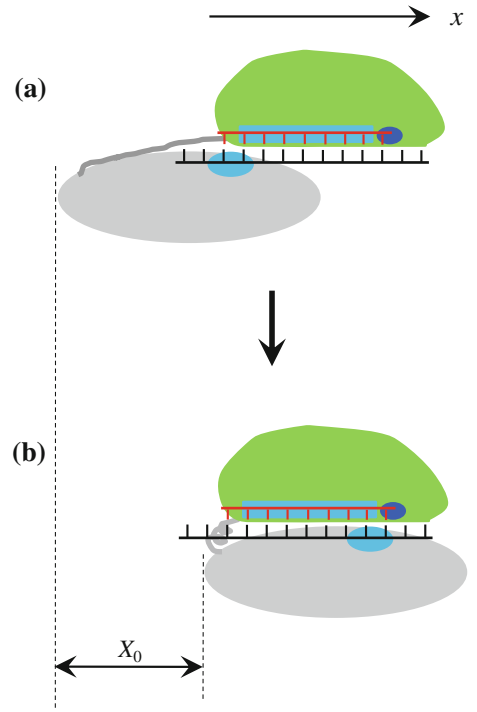

Fig. 4 Schematic illustrations of the initiation of primer-dimer synthesis. **a** After synthesis of unit-length primer, the p58 subunit is still binding to the original binding position on the DNA template. **b** After the p58 subunit dissociates from the original binding position, the elastic force drives the p58 subunit returning to the equilibrium position relative to the p49 subunit, where the DNA-binding site rebinds to the DNA template

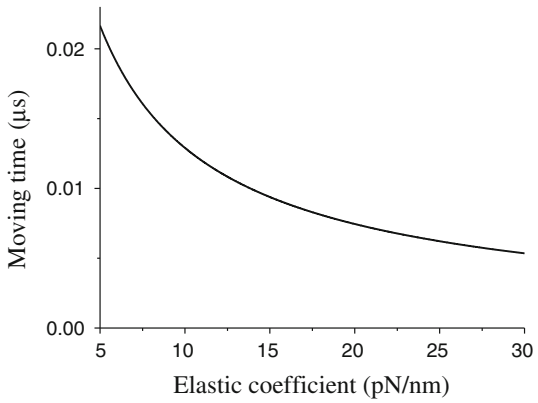

Fig. 5 The mean time for the p58 subunit to move from the position with the maximum deviation distance from the p49 subunit to the equilibrium position upon the dissociation of the p58 subunit from the DNA template

that the primase alone (p49–p58 complex) induces the negative regulation of further primase activity after synthesis of a unit-length primer, which is consistent with the experimental data (Arezi et al. 1999). Moreover, our results indicate that the affinity of the p58 subunit for the DNA should be $E_{p58} > 24.7\ k_BT$ and the RNA-binding site in the p49 subunit could bind $m \geq 6$ RNA bases, with an affinity $E_{p49} = mE \geq 25.8\ k_BT$.

Discussion

In this work, we present a model for processive primer extension by eukaryotic DNA primase, which corresponds to the hinge model (from "closed" to "open" hinge) described in Kuchta and Stengel (2010). It is noted that the present model is somewhat similar to that proposed for telomere elongation by telomerase (Xie 2009, 2010), another type of eukaryotic nucleic acid polymerases capable of DNA synthesis de novo. In the present model for the primase, the p58 subunit binds fixedly to the template while the catalytic p49 subunit translocates along the template. By contrast, in the model for the telomerase, the RNA-binding domain (TRBD) and/or N-terminal extension (TEN) of telomerase reverse transcriptase (TERT) binds fixedly to the template, while the catalytic reverse transcriptase (RT) domain of TERT translocates along the template.

The p49 subunit may bind RNA-DNA duplex

In the model, based on the experimental evidence that only the p58 subunit of the p49–p58 primase complex reacted with the DNA (Arezi et al. 1999), we assume that the p49 subunit binds the RNA strand of the RNA-DNA duplex. Interestingly, it is noted that, if we assume that the p49 subunit binds the RNA-DNA duplex, all the theoretical results would be the same as those that have been obtained up to now by assuming that the p49 subunit binds only the RNA strand of the RNA-DNA duplex.

As is known, decreasing the temperature or increasing the number of G:C base pairs in the RNA-DNA duplex increases the stability of the RNA-DNA duplex. Thus, from the assumption that the p49 subunit binds the RNA-DNA duplex, we deduce that decreasing the temperature or increasing the number of G:C base pairs in the RNA-DNA duplex increases the binding affinity of the p49 subunit for the RNA-DNA duplex, which in turn decreases the amount of the generated abortive RNA primers. This is consistent with the experimental data (Kuchta and Stengel 2010). However, these experimental data seem not to be readily explained by assuming that the p49 subunit binds only the RNA strand of the RNA-DNA duplex. Thus, in order to be consistent with all the available experimental data, we should modify the assumption that the p49 subunit binds only the RNA strand of the RNA-DNA duplex as follows: the p49 subunit binds the RNA-DNA duplex via interacting mainly with the RNA strand of the RNA-DNA duplex.

On interaction of the p49 subunit with ssDNA

Experimental data showed that both prior to and after primer synthesis, only the p58 subunit of the p49–p58 primase complex reacted with DNA (Arezi et al. 1999). However, the experimental data showed that the isolated p49 subunit also reacted with ssDNA (Arezi et al. 1999). A possible explanation for these experimental data is as follows. The interaction of the p49 subunit with the p58 subunit in the p49–p58 primase complex could make the DNA binding residues in the p49 subunit become extremely nonreactive, as suggested by Arezi et al. (1999).

However, if we assume that the p49 subunit in the p49–p58 primase complex, besides binding the RNA strand or mainly the RNA strand of the RNA-DNA duplex, also has an affinity for the unpaired base and/or the sugar-phosphate backbone of the ssDNA, the model presented in this work would also be applicable. For this case, U_0 in Eqs. 1 and 2 denotes, instead of only nE, the summary of the binding affinity for the primer, nE, and the binding affinity for the ssDNA, E_{ssDNA}, where E_{ssDNA} is a constant independent of the number of the incorporated RNA bases. Nevertheless, it is easily seen that this would give the same results as those presented in this work.

Concluding remarks

A model is presented for processive primer extension by eukaryotic DNA primase. Based on the model, the dynamic behaviors of the primase activity are theoretically studied. The results are in agreement with the experimental data. Moreover, it is predicted that after the generation of the dinucleotide and during the processive primer extension, the NTP-binding rate is estimated to be 80 μM^{-1} s^{-1}; the affinity of the p58 subunit for the DNA is about $E_{p58} > 24.7\ k_BT$; and the RNA-binding site in the p49 subunit can bind $m \geq 6$ RNA bases, with an affinity of about $E = 4.3\ k_BT$ per RNA base and a total affinity of about $E_{p49} = mE \geq 25.8\ k_BT$.

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Appendix 1: Dissociation time distribution of primase from DNA template during initiation of primer synthesis

Similar to Eq. 1, during initiation of primer synthesis, the movement of primase along the direction (the y direction) perpendicular to the single-stranded DNA (ssDNA) template is described by the following Langevin equation

$$\Gamma \frac{\mathrm{d}y}{\mathrm{d}t} = -\frac{\partial [U_{\text{primase}}(y)]}{\partial y} + \xi_y(t), \tag{10}$$

where $U_{\text{primase}}(y) = U_{p58}^{(0)}[\exp(-2y/d) - 2\exp(-y/d)]$ is the interaction potential of the p58 subunit with the ssDNA, with $U_{p58}^{(0)}$ representing the binding affinity.

As in the main text (see Fig. 2b), the numerical results by solving Eq. 10 show that the dissociation time of the primase from the ssDNA template before the binding of two NTPs also has a single-exponential distribution, i.e., $f_d^{(2)}(t) = C\exp(-t/T_d^{(2)})$, where $T_d^{(2)}$ is the mean dissociation time of the primase from the DNA.

Appendix 2: Probability for the primase to generate a dinucleotide primer

As shown in "Appendix 1," the dissociation time of the primase from the ssDNA template before the binding of two NTPs has a single-exponential distribution, i.e., $f_d^{(2)}(t) = \exp(-t/T_d^{(2)})/T_d^{(2)}$.

Experimental evidence indicated that, under conditions of high NTP concentration, primer synthesis would occur at the first potential synthesis site to which primase binds, implying that the primase becomes bound strongly to the DNA after two NTPs bind (Kirk et al. 1997). Thus, for approximation, it is considered that the primase has a negligible probability to dissociate from the DNA template after two NTPs bind. The time distribution for binding of two NTPs would have a single-exponential form, i.e., $f_b^{(2)}(t) = \exp(-t/T_b^{(2)})/T_b^{(2)}$, provided that either of the following two conditions is satisfied. One is that the bindings of the two NTPs are independent and thus $T_b^{(2)}$ is the longer one of the two mean NTP-binding times. The other one is that the bindings of two NTPs are dependent, with the binding time for one NTP being much longer than that for another one. Thus, $T_b^{(2)}$ is also approximately the longer one of the two mean NTP-binding times. Here we assume the single-exponential time distribution for the binding of two NTPs.

In order to derive the equation of the probability for the primase to generate a dinucleotide primer, $P^{(2)}$, we first adopt the dissociation-time distribution of the primase from the ssDNA template having a function $f_d^{(2)}(t) = \delta(t - T_d^{(2)})$ after it binds to the ssDNA template. For this δ-function distribution, $P^{(2)}$ can be obtained from $P^{(2)} = \int_0^{T_d^{(2)}} f_b^{(2)}(t)\,\mathrm{d}t = \int_0^{T_d^{(2)}} \exp(-t/T_b^{(2)})/T_b^{(2)}\,\mathrm{d}t$, i.e.,

$$P^{(2)} = \left[1 - \exp\left(-\frac{T_d^{(2)}}{T_b^{(2)}}\right)\right]. \tag{11}$$

For the real dissociation-time distribution, $f_d^{(2)}(t) = \exp(-t/T_d^{(2)})/T_d^{(2)}$, using Eq. 11, $P^{(2)}$ can be obtained from $P^{(2)} = \int_0^\infty [1 - \exp(-t/T_b^{(2)})] \exp(-t/T_d^{(2)})/T_d^{(2)}\, dt$, which has the form

$$P^{(2)} = \frac{T_d^{(2)}}{T_b^{(2)} + T_d^{(2)}}. \quad (12)$$

where $T_b^{(2)} = (k_b^{(2)}[\mathrm{NTP}])^{-1}$, with $k_b^{(2)}$ being the NTP-binding rate.

Appendix 3: Probability for the primase to incorporate the (n + 1)th RNA base that is connected to the RNA primer of n bases ($n \geq 2$)

Using the similar procedure to that used in "Appendix 2," the probability for the primase to incorporate the (n + 1)th RNA base that is connected to the RNA primer of n bases ($n \geq 2$) is obtained as follows

$$P_n = \frac{T_{dn}}{T_{b(n+1)} + T_{dn}}, \quad (13)$$

where $T_{b(n+1)} = \left(k_{b(n+1)}[\mathrm{NTP}]\right)^{-1}$ is the mean NTP-binding time for the (n + 1)th NTP, with $k_{b(n+1)}$ being the NTP-binding rate, and T_{dn} is the mean dissociation time of the p49 subunit from the RNA primer of n bases. In Eq. 13, it is implicitly considered that the p49 subunit has a zero probability to dissociate from the RNA primer after NTP binds. In fact, available experimental data indicated that, at saturating NTP concentration, the p49 subunit can still dissociate from the RNA primer (Kuchta et al. 1990). Denoting $p_d^{(n)}$ the dissociation probability of the p49 subunit from the RNA primer of n bases after NTP binds, then from Eq. 13 the probability for the primase to incorporate the (n + 1)th RNA base that is connected to the RNA primer of n bases ($n \geq 2$) is rewritten as

$$P_n = p_p^{(n)} \frac{T_{dn}}{T_{b(n+1)} + T_{dn}}, \quad (14)$$

where $p_p^{(n)} = 1 - p_d^{(n)}$ is the probability for the primase to complete the incorporation of the (n + 1)th RNA base after the NTP binds.

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