Kumar & Patel, 1997). As has been done previously, the data can be fit well by the equation:

$$[\mathrm{RNA}] = A_{\mathrm{Burst}}(1 - e^{-k_{\mathrm{fast}}t}) + k_{\mathrm{ss}}t \qquad (1)$$

The steady-state parameter suggests a unimolecular rate constant of $k_{ss}/[RP]$ of 0.23 s^{-1} at 25 °C (given that DNA is limiting, at 3 μM). It was previously proposed that the rate-determining step in the steady-state synthesis of short RNA products is the release of product RNA and/or polymerase recycling, with a first order rate constant of about 0.15 s^{-1} (Jia & Patel, 1997a,b). An immediate problem with this fit, however, is that the burst amplitude (5.2 μM) is larger than the limiting DNA concentration (3.0 μM). In this simple model, the burst should correspond to an initial single turnover.

Equation (1) does not derive from a specific kinetic model. In order to more clearly interpret the kinetic profile, we instead fit the data to numerically integrated solutions of simple kinetic models. Recognizing that open complex formation is not rate determining in this system (Jia *et al.*, 1996; Újvári & Martin, 1996), we initially attempted to fit the above data to an equation of the form:

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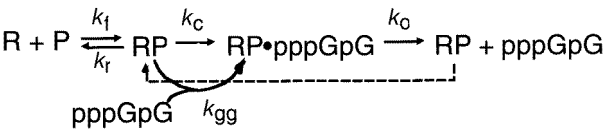
where RNA polymerase (R) combines with the promoter DNA (P) to form an initial binary complex (RP). Under conditions in which the concentration of GTP does not change substantially during the course of the reaction, the binding of two molecules of GTP and the catalysis of phosphodiester bond formation can be treated as pseudo-first order process (represented by a concentration-dependent k_c). The best fit of the data in Figure 1 to the above kinetic mechanism is shown as a broken line. The parameters describing binding have been previously determined ($k_f = 126$ μM^{-1} s^{-1} and $k_r = 0.20$ s^{-1}), and since that reaction is fast and lies far to the right under these conditions, the fit shows only a minor dependence on them (Jia *et al.*, 1996; Újvári & Martin, 1996). Fixing those parameters, the best-fit values for k_c (2.3(±1.0) s^{-1}) and k_o (0.35(±0.03) s^{-1}) are comparable to those obtained previously, 3.5 s^{-1} and 0.15 s^{-1}, respectively (Jia & Patel, 1997b). Careful inspection of the curve and the residuals plot, however, shows that equation (2) does not accurately represent the observed data. Similar kinetic models were tried, including models in which the enzyme-DNA complex initially formed must undergo a transition to an activated state. However, these models were unable to provide a fit significantly better than that provided by equation (2).

In considering alternative models, we can exclude from consideration possible product inhibition by accumulated pyrophosphate, since the reported K_i for PP_i is 830 μM (Guajardo & Sousa, 1997). However, it is well known that T7 RNA polymerase can initiate with a dinucleotide as an initial substrate (Jia & Patel, 1997b; Moroney & Piccirilli, 1991), so we reasoned that as the product dinucleotide accumulates, it might serve as a competitive inhibitor of initiation. This results in inhibition only under conditions of dinucleotide synthesis, in the synthesis of longer products such rebinding is productive, but nevertheless cannot be ignored in kinetic treatments. The continuous line in Figure 1 shows the best fit of the data to the following equation, which incorporates product inhibition explicitely:



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In this case, at a saturating concentration of GTP (800 μM, see below), the best fit values for k_c (1.3(±0.2) s^{-1}) and k_o (1.0(±0.3) s^{-1}) are comparable, and the apparent bimolecular rate constant for product rebinding (k_{gg}) is 0.21(±0.07) μM^{-1} s^{-1}, such that this step becomes significant within a single turnover (at 3 μM pppGpG, the pseudo-first order rate constant for this step becomes 0.6 s^{-1}). The ratio k_o/k_{gg} (4.8 μM) provides a measure of the equilibrium dissocation constant K_d for binding of dinucleotide to the polymerase-promoter complex. This low value explains why product inhibition becomes significant even at relatively low product concentrations, since the K_d values for GTP binding are 20-200 times higher (Jia & Patel, 1997a).

Direct measurement of dinucleotide consumption

In order to measure more directly kinetic parameters for the incorporation of dinucleotide described by the parameter k_{gg}, we carried out transcription in the presence of ATP, CTP, UTP and the dinucleotide pppGpG (producing a five base runoff transcript). The kinetic curves shown in Figure 2(a) follow simultaneously the production of the runoff transcript and the consumption of dinucleotide under conditions of low dinucleotide concentration, where the binding represented by k_{gg} is rate limiting. For this reaction, the estimated second order rate constant for dinucleotide consumption (k_{app} divided by the limiting concentration of enzyme-DNA complex, 0.02 μM)