Hypothesis

RNA POLYMERASE: A MODEL FOR ROTATIONAL TRANSLOCATION

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1. Introduction

DNA-dependent RNA polymerase is the enzyme involved in the transcription process. During transcription, ribonucleoside triphosphate (NTP) monomers are polymerized into a transcript (RNA) with sequence and length specified by a template DNA molecule.

This transcription process has two chemical mechanistic aspects:

(i) The selection of the proper NTP as a substrate;
(ii) The catalysis of the formation of a phosphodiester bond between the 3′-hydroxyl group of the ribose moiety at the terminus of the growing chain and the 5′ α-phosphate of the incoming NTP monomer.

After selection and catalysis, translocation of the newly formed 3′→5′ phosphodiester bond frees the active site for the next incoming monomer. Translocation poses mechanistic problems for all template-directed biological polymerizations. Woese [1] has considered in detail the translocation problem for protein synthesis, and has argued for the necessity of functionally symmetric active sites on the ribosomes. His model is operationally 'a reciprocating ratchet mechanism'. Our concerns here are the molecular details of a model for translocation in the transcription process.

We present a model for translocation together with suggestions for its experimental testing. The novel features of the model include:

(i) Two functionally equivalent active sites – each site consists of a binding locus and a catalytic locus. The sites have 2-fold rotational symmetry with respect to the relevant marker atoms of the cyclic compound *bis* 3′→5′ di-ribose 5′-phosphate (fig.1).
(ii) Functional symmetry – the translocation of the growing transcript from one registry to the next is accomplished in 180° rotational increments of the enzyme in its downstream course along the DNA template. Occupancy alternates between each of the symmetrically equivalent active sites.

In the following discussion we present the topography of the active site and the dynamics of translocation.

2. Formulation of the topography of the active site

Each of the two functional active sites contains two binding loci which position the incoming substrate ribose moiety proximal to the product terminus ribose moiety. When so positioned, the α-phosphate of the incoming ribose and the 3′-hydroxyl of the product terminus ribose are within bonding distance. The surface of the enzyme's active site at these loci (3′-hydroxyl and α-phosphate) plays a catalytic role. This aspect of the model, in itself, is not novel and is consistent with the data and conclusions in [2–4].

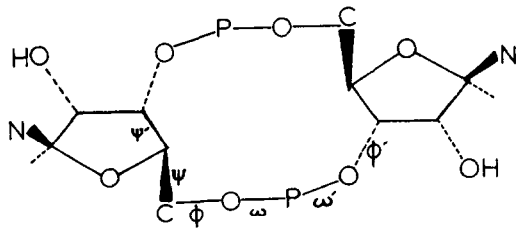

Fig.1. Symmetric representation of *bis* 3′→5′ di-ribose 5′-phosphate. Conformational rotation angles are labelled for bonds of interest. (N) = hydroxyl for ribose *or* purine or pyrimidine base for nucleotides.

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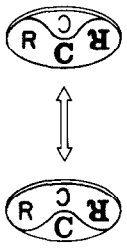

Fig.2. The symmetry of the two functionally active sites. The equivalent active sites (shaded areas) of the enzyme which are alternately used during rotationaly translocation. For either site, one ribose binding locus is occupied by the product terminus while the other is occupied by the ribose of the incoming monomeric NTP. R or R = ribose-binding locus; C or C = catalytic locus.

Our novel idea is the suggestion that there exist two equivalent active sites related by a 2-fold rotational symmetry. These active sites are used alternately and contain overlapping regions on the enzyme surface, as depicted in fig.2.

The substrate ribose binds in the 3′-*endo* conformation, as indicated by our earlier kinetic studies [5,6]. These results are summarized in table 1. From the data in table 1 we can conclude that when the 3′-hydroxyl of the incoming substrate is positioned above the catalytic locus of the unoccupied active site, its 2′-hydroxyl will be bound in the corresponding binding locus.

We have modelled the enzyme's active-site topography by considering bond lengths and angles taken from the cyclic compound *bis* 3′→5′ di-ribose 5′-phosphate. This compound gives the minimal coordinates for a phosphodiester bond and has a symmetry which allows the next phosphodiester bond to be included as well. With this cyclic cognate held planar with respect to the ribose moieties, the purine or pyrimidine bases project above the plane, and they are available for interaction with the DNA and/or other parts of the enzyme surface. Fixed in this configuration, the conformation of the backbone bonds $\{\Psi', \Psi, \phi, \omega', \phi'\}$ is $\{g^+, g^+, t, g^+, g^+, t\}$. The molecule is shown in fig.1 and defines the symmetry of the surface of the active sites.

3. Sequence of events for binding, catalysis and translocation

In our model, binding, catalysis and translocation are presented such that the enzyme alternately uses each of the two active sites during the course of the polymerization. These events are depicted in fig.3 and are described hereafter. The binding of a catalytically competent substrate involves positioning the 2′-hydroxyl of ribose (3′-*endo* conformation) at the binding locus with the α-phosphate in one catalytic locus and the 3′-hydroxyl poised above the other (unoccupied) catalytic locus. When so positioned, phosphodiester bond formation is catalysed between the α-phosphate of the incoming substrate ribose and the

Table 1
Kinetic inhibition studies summary for pentose-5-triphosphates and/or nucleopentose-5′-triphosphates

D-Pentose	2- and 3-Hydroxyl conformation	K_i (μM)	
		Pentose · P_3	Nucleopentose · P_3
Ribose [5]		21	13 (K_m)
2–*d*–Ribose [5]		26	2800
Arabinose [6]		60	>5000
Xylose [7]		–	37
3–*d*–Ribose [8]		–	18
2–*d*–2 NH_2 Ribose [9]		–	>2000
3–*d*–3 NH_2 Ribose [9]		–	2

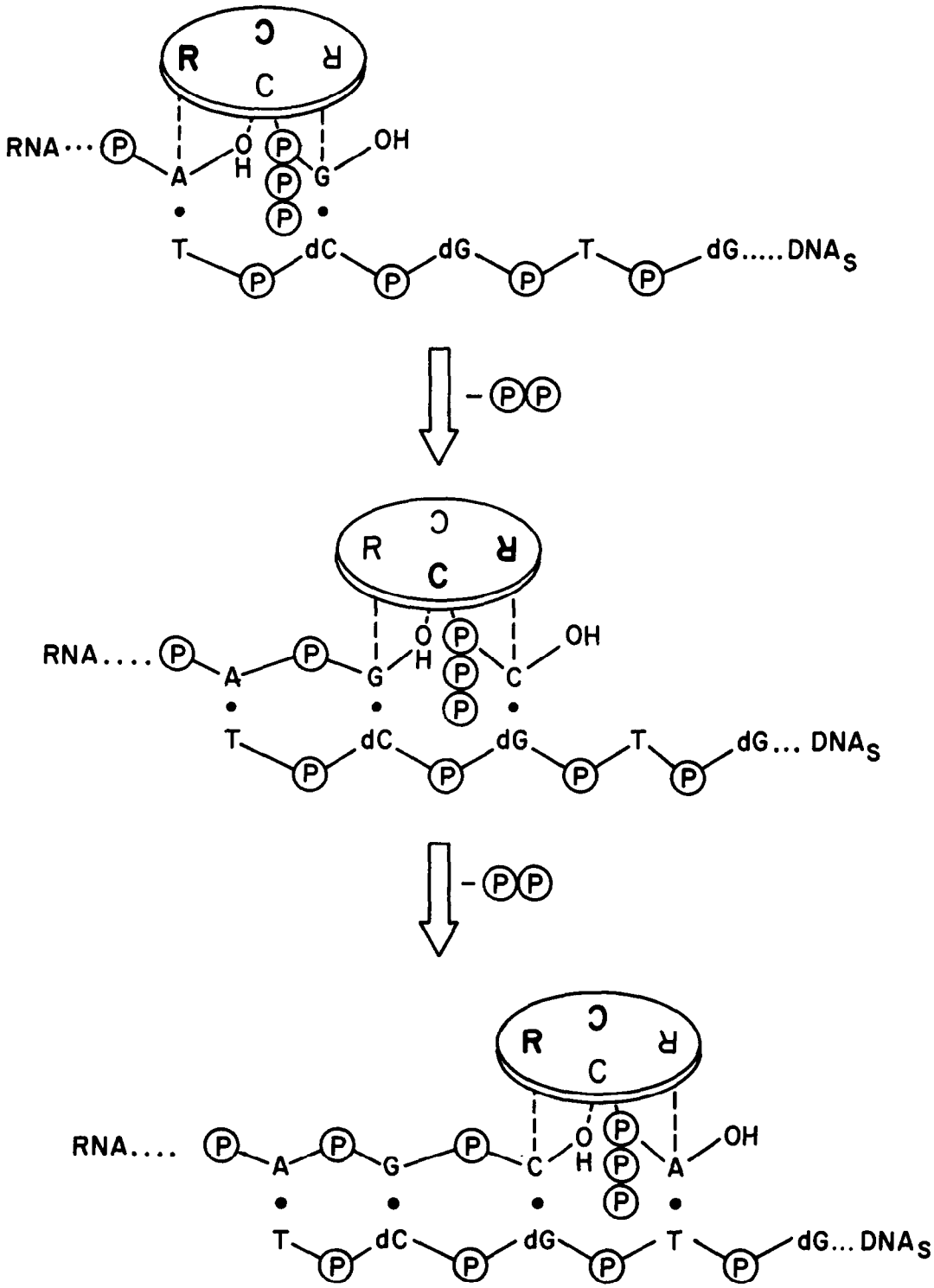

Fig.3. Translocation during three successive substrate additions. R or R = ribose-binding loci for either the product terminus or an incoming substrate. C or C equivalent catalytic loci that catalyse the formation of the phosphodiester bond.

3′-hydroxyl of the ribose bound at the product terminus site.

The phosphodiester bond so formed would be in the eclipsed conformation with respect to the C(5′) and O(3′) atoms about the O(5′)–phosphorus bond. Upon completion of phosphodiester bond formation, the ribose is displaced from the former product terminus site; at the same time, the 3′-hydroxyl of the new product terminus nucleotide is inserted into the unoccupied catalytic locus. Three events occur which energetically favor this displacement:

(i) Rotation about the O(5′)–phosphorus bond by 90° to relieve the strain of the eclipsed conformation.

(ii) Change of the ribose conformation from 3′-*endo* to 2′-*endo* with insertion of the 3′-hydroxyl into the catalytic locus. The 2′-hydroxyl remains bound at its original binding locus on the enzyme surface (see fig.4A,B).

(iii) Binding of the base substituent of C(1′) at the enzyme surface.

These three events: rotation about the O(5′)–phosphorus bond; the conformational change of ribose from 3′- to 2′-*endo;* and the new base–enzyme interaction; are proposed as the driving forces that destabilize and expel the ribose from the former product terminus binding site. The 3′-hydroxyl of the new product terminus ribose moiety is positioned at the catalytic locus ready to react with the α-phosphate of the incoming substrate nucleotide.

The above three events constitute our proposed mechanism for translocation. As a consequence of these events, at each step the alternate ribose binding locus is made available and the incoming ribose moiety

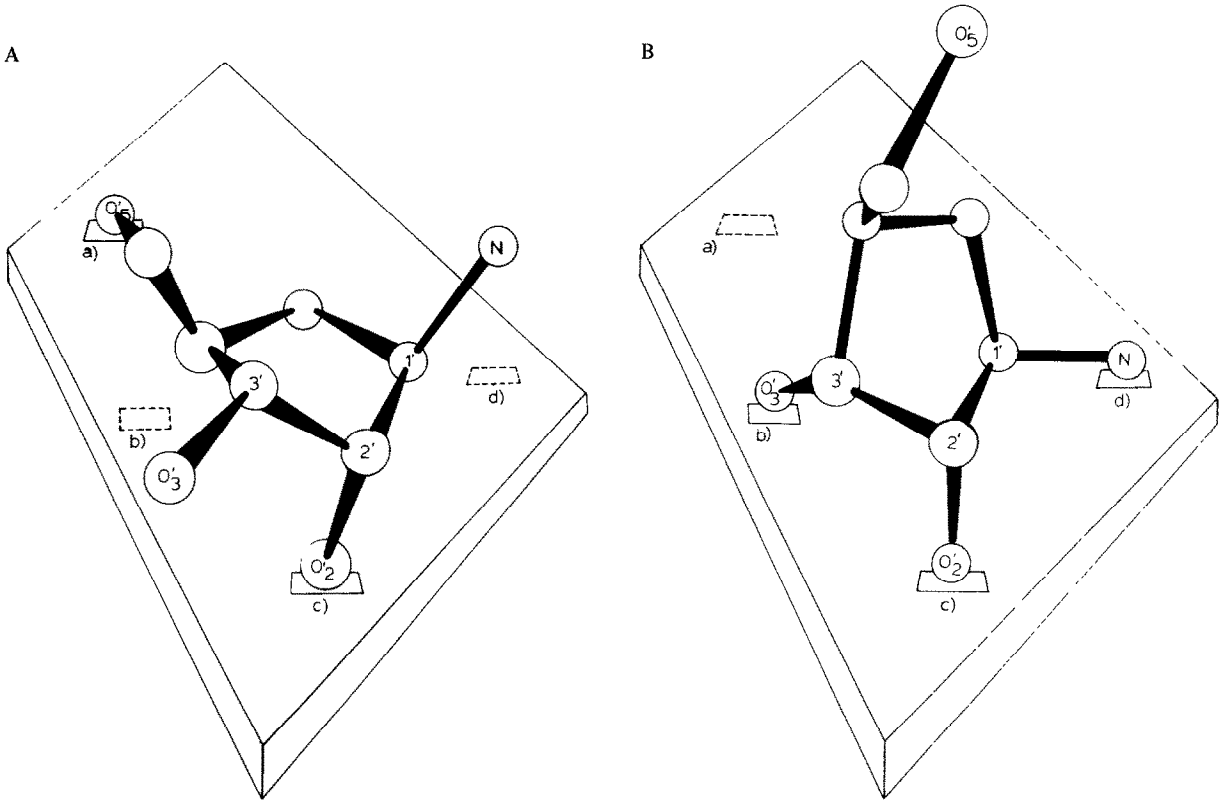

Fig.4. The portion of the active site surface occupied by the 3′- and 2′-*endo* conformational states or the ribose substrate moiety. Relevant contact areas are labeled a, b, c and d. (A) Prior to formation of the phosphodiester bond, the substrate is bound in the 3′-*endo* conformation. The O(5′) is a marker for the α-phosphate which is bound at area (a). The 2′-hydroxyl is bound at area (c) but areas (b) and (d) are unoccupied. (B) After phosphodiester bond formation the new product terminus ribose in the 2′-*endo* conformation. The 3′-hydroxyl is now bound to area (b), the base (N) is bound at area (d) and the 2′-hydroxyl is still bound at area (c). Note that area (a) is now unoccupied and that the phosphorus atom of the newly formed phosphodiester bond is located well above the surface of the active site.

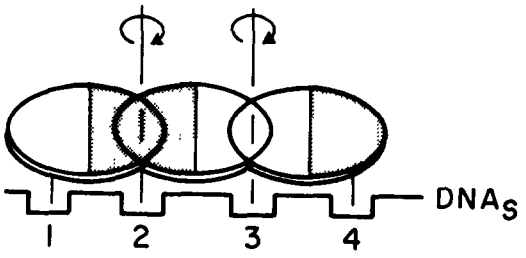

Fig.5. The dynamics of translocation. The rotational movement of the active site surface of the enzyme as it progresses along the sense strand of DNA.

binds there. Upon binding the α-phosphate is positioned at the catalytic locus already occupied by the 3′-hydroxyl of the product terminus ribose.

A pictorial representation of the relative movement of the enzyme active sites surface along the longitudinal axis of the single strand region of the DNA is presented in fig.5. The enzyme rotates along the DNA template using each active site in turn.

This mode of translocation is attractive because it provides a chemical mechanism for translocation. The chemistry so described affords an energetically feasible path for the change of registry.

Our suggestion that there are two functionally identical active sites is a testable requirement of this model. An active site reagent added during the elongation phase would have a 50:50 chance of derivatizing either one (but only one) of the two active sites.

The topology of the active sites suggested by the model serves as a blueprint for the design of unusual non-competitive inhibitors which can simultaneously bridge both active sites in the absence of substrates. These same non-competitive inhibitors should be ineffective during elongation since only one of the active sites will be accessible at any given time. These studies can serve to test the model. We are currently synthesizing several potential active-site agents and bridging compounds in order to test the predictions of this model.

The model for rotational translocation described here may have application to other template-directed polymerization processes of biological interest.

Acknowledgements

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