DNA Polymerase III Holoenzyme

COMPONENTS, STRUCTURE, AND MECHANISM
OF A TRUE REPLICATIVE COMPLEX

Charles S. McHenry
From the Department of Biochemistry, Biophysics, and Genetics, University of Colorado Health Science Center, Denver, Colorado 80262

The DNA polymerase III holoenzyme is the replicative polymerase of Escherichia coli, responsible for synthesis of the majority of the chromosome (for a more extensive review, see Ref. 1). The replicative role of the enzyme has been established through both biochemical and genetic criteria. Holoenzyme\(^1\) has been biochemically defined and purified using natural chromosomal assays. Only the holoenzyme form of pol III will efficiently replicate the DNA of single-stranded bacteriophages \textit{in vitro} in the presence of other known E. coli replicative proteins (2–4). Similarly, only the holoenzyme will function in the replication of bacteriophage \(\lambda\), plasmids, and DNA containing the \(E. coli\) replicative origin (5–8). Genetic studies also support assignment of the major replicative role to the holoenzyme. The holoenzyme appears to contain up to 10 different subunits (Table I). Temperature-sensitive, conditionally lethal mutations in four replication genes can be correlated with defects in five of the subunits of holoenzyme; the genes for the remaining five subunits remain unknown (Table I).

There are at least three distinct polymerases in \(E. coli\), yet only holoenzyme appears to play a major replicative role. What are the special features of the holoenzyme that confer its unique role in replication? Work to date suggests that an ability to rapidly elongate in a highly processive mode at physiological levels of salt, to utilize a long single-stranded template coated with the single-stranded DNA-binding protein, to jump over obstacles created by annealed oligonucleotides, to interact with other proteins of the replicative apparatus, and to coordinate replication through an asymmetric dimeric structure are critical to its unique functions. Many of these features are conserved between bacterial and eukaryotic systems, suggesting that insight gained through studies with the holoenzyme may be transferable to a variety of life forms.

\textit{Multiple DNA Polymerase III Forms}

The holoenzyme can be biochemically resolved into a series of successively simpler forms (Table II). Having these multiple forms available has facilitated assignment of functions to individual holoenzyme subunits and assemblies. While it is possible that some polymerase subassemblies may exist in the cell free by themselves, it is unlikely that they make significant synthetic contributions \textit{in vivo} given their extreme sensitivity to physiological ionic strengths (42, 43).

\textit{Processivity}

Studies of the processivities of the multiple pol III forms have revealed contributions of individual subunits (Table II) (38, 40, 41). The multiple forms of pol III exhibit strikingly different processivities. The processivity of the core pol III is nearly distributive under physiological ionic strength. Processivity is enhanced by addition of the \(\gamma\) subunit to form pol III', pol III' achieves maximum processivity in the presence of physiological concentrations of spermidine, an agent that inhibits the core pol III. Addition of the \(\gamma\) complex\(^2\) (\(\gamma, \delta, \delta', \psi, \text{and } \chi\)) to pol III' to form pol III'\(^*\) further increases processivity in the presence of single-stranded DNA-binding protein. The holoenzyme is exceedingly processive, having the capability of remaining stably bound to a template for 30–40 min, the time required for replication of the entire \(E. coli\) chromosome (1, 38, 39, 44). The processivity of the proofreading exonuclease responds like the elongation activity to the addition of holoenzyme auxiliary subunits (45). Thus, a progression in processivities is observed that parallels the structural complexity of the corresponding enzyme form.

\textit{Initiation Complex Formation and a Kinetic Barrier to Polymerase Cycling}

To achieve high processivity, the holoenzyme requires ATP and primed DNA to form a stable initiation complex (2, 38). Initiation complexes can be isolated by gel filtration and upon addition of dNTPs form a complete RFII in 10–15 s (2, 27). \(\beta\) (and presumably the remaining holoenzyme components) remains stably associated with both initiation and termination complexes (RFII) for at least 30 min (44). This observation revealed a problem in the \textit{in vitro} system. Okazaki fragments are made on the lagging strand of the replication fork each second at 37 °C. This requires the lagging strand polymerase to bind to a primer, synthesize a 1000-nucleotide Okazaki fragment, dissociate, and bind to the next primer each second. The 30 min we observe for release of holoenzyme \textit{in vitro} is considerably slower than the fraction of a second required \textit{in vivo}.

At the time that we were struggling with the issue created by the kinetic barrier to recycling, we discovered that pol III' was dimeric and inferred that the holoenzyme was as well (18). This had important implications regarding the cycling problem when considered in the context of Bruce Alberts' model for cycling during bacteriophage T4 replication. Alberts (46, 47) proposed that at the replication fork, T4 polymerase formed a dimer of leading and lagging strand polymerases.\(^3\) This would provide a mechanism for retargeting the lagging strand polymerase to the next primer for Okazaki fragment synthesis rather than permitting the polymerase to diffuse to another fork. It occurred to us that the Alberts model might

\(\text{\textsuperscript{1}}\) The abbreviations used are: holoenzyme, DNA polymerase III holoenzyme; pol III, DNA polymerase III; ATPyS, adenosine 5'-O-(thiotriphosphate).

\(\text{\textsuperscript{2}}\) The \(\gamma\) complex was initially purified as an activity that permitted reconstruction of holoenzyme using core DNA polymerase III and the \(\beta\) subunit (4). With improvements in gel systems, added subunits became visible in holoenzyme (13) and the \(\gamma\) complex (29). The five-subunit structure of the \(\gamma\) complex originally purified in 1978 (4, 29) was identical to the five-subunit \(\gamma\) complex described in 1988 (30). In this minireview, I use the original nomenclature for the \(\gamma\) complex to refer to the five-subunit complex.

\(\text{\textsuperscript{3}}\) Subsequent to the proposal of the Alberts dimeric T4 model, the Kornberg laboratory (48, 49) also proposed that the proteins at the \(E. coli\) replication fork might coordinate replication of the leading and lagging strand through a dimeric replicative complex. This model, however, did not entail an asymmetric polymerase with functionally or structurally distinguishable leading and lagging strand halves.
provide a solution to the *E. coli* cycling problem and an explanation for our observations. If the replicative complex were dimeric, then an opportunity for communication between polymerase halves would be provided. To guarantee a properly assembled complex continuously associated with the replication fork, the lagging strand polymerase might only be able to dissociate when the leading strand polymerase is in a productive elongation conformation. If this were true, then dissociation of the lagging strand polymerase might be slow when a uniform population of single-stranded templates were used since they would all be completed at nearly the same time. A mechanism permitting communication between the leading and lagging strand polymerases might be useful in coordinating leading with lagging strand replication, as I have discussed elsewhere (50).

**The Asymmetric Dimer Hypothesis**

To further investigate initiation complexes, we explored the use of ATP analogs. ATP\(\gamma\)S was found to substitute for ATP in initiation complex formation, but only partially. In spite of a greater efficacy at low nucleotide concentrations, ATP\(\gamma\)S only supported formation of one-half as much complex as ATP. The same effect was observed upon reversal of the reaction. ATP\(\gamma\)S caused one-half of the initiation complexes to dissociate; ATP exhibited no effect. Based on this observation we first proposed the asymmetric dimer hypothesis, a modification of previous notions about dimeric polymerases (51, 52). We suggested that ATP\(\gamma\)S might be revealing a functional asymmetry in the holoenzyme consistent with distinct leading and lagging strand polymerase, with ATP\(\gamma\)S having differential effects on the two halves. Such an asymmetry would be consistent with the asymmetric functional requirement of polymerases at replication forks. The leading strand polymerase, once associated with the replication fork, need not dissociate until the entire chromosome is replicated, a process that takes approximately 40 min in *E. coli*. In contrast, the lagging strand polymerase needs to dissociate from the completed product, reassociate with the next primer, and synthesize an entire Okazaki fragment each second in the cycle depicted in Fig. 1. It would appear to be advantageous for a cell to contain an asymmetric dimeric polymerase with an extremely processive leading strand polymerase and a lagging strand polymerase with modified properties that permit rapid cycling.

Although it is attractive and has won widespread acceptance, the asymmetric dimer hypothesis remains unproven. It has not yet been rigorously demonstrated that the holoenzyme has two polymerase entities that can function in concert at a replication fork. Even physical characterizations of holoenzyme as a dimer (53) are subject to question, since they did not contain corrections for the high frictional coefficients of elongated polymerases that have been observed in simpler forms (18). However, considerable supportive data for the hypothesis have been obtained. One prediction of the model is that the enzyme is not only dimeric, but interactive, so that the leading and lagging strand polymerases can communicate during coordinated replication. Strong positive cooperativity has been demonstrated between the ATP binding sites for initiation complex formation (29).

Additional support for the asymmetric dimer model was derived from studies of the \(\gamma\) and \(\tau\) subunits of the pol III holoenzyme. \(\tau\) and \(\gamma\) are both products of the *dnaX* gene (16, 17). We first proposed that translational frameshifting accounted for generation of these two subunits from one gene and, based on conjecture and sequence data, proposed a potential site (1). The experimental results from our laboratory and others verified a frameshifting mechanism, but at the sequence AAAAAG followed by a strong hairpin, similar to the frameshifting site in retroviruses (24, 54–56). The AAAAAG sequence had been identified earlier as a "shifty" sequence in model constructs (57). The sequence of *dnaX* suggested a tight DNA binding domain in the carboxyl terminus of the gene within sequences contained in \(\tau\) but not \(\gamma\) (58). This finding, coupled with the knowledge that \(\tau\), but not \(\gamma\), binds to the pol III core and forms a polymerase of increased processivity, led to the suggestion that \(\tau\) may be in the leading strand half conferring high processivity, and \(\gamma\) in the lagging strand half permitting more rapid cycling, mediated by looser \(\gamma\)-template interactions (58, 59). In the future, it will be interesting to learn whether frameshifting is regulated to guarantee an appropriate ratio of leading and lagging strand components.

Recent work has provided further support for the asym-

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### Table I

*Subunits of the DNA polymerase III holoenzyme*

<table>
<thead>
<tr>
<th>Subunit</th>
<th>(M_r)</th>
<th>Structural gene</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\alpha)</td>
<td>129,900</td>
<td><em>dnaE</em></td>
<td>9–14</td>
</tr>
<tr>
<td>(\tau)</td>
<td>71,100</td>
<td><em>dnaX</em></td>
<td>15–20</td>
</tr>
<tr>
<td>(\gamma)</td>
<td>47,500</td>
<td><em>dnaX</em>(^*)</td>
<td>4, 16, 17, 19–24</td>
</tr>
<tr>
<td>(\beta)</td>
<td>40,600</td>
<td><em>dnaN</em></td>
<td>4, 25–28</td>
</tr>
<tr>
<td>(\delta)</td>
<td>34,000</td>
<td>(\delta)</td>
<td>4</td>
</tr>
<tr>
<td>(\delta')</td>
<td>31,000</td>
<td>(\delta')</td>
<td>29–31</td>
</tr>
<tr>
<td>(\epsilon)</td>
<td>27,500</td>
<td><em>dnaQ</em> (mucD)</td>
<td>32–37</td>
</tr>
<tr>
<td>(\psi)</td>
<td>14,000–16,000</td>
<td>(\psi)</td>
<td>1, 29–31</td>
</tr>
<tr>
<td>(\chi)</td>
<td>12,000–14,000</td>
<td>(\chi)</td>
<td>1, 29–31</td>
</tr>
<tr>
<td>(\theta)</td>
<td>10,000</td>
<td>(\theta)</td>
<td>13</td>
</tr>
</tbody>
</table>

*Formerly *dnaZ*.

### Table II

*DNA polymerase III forms*

<table>
<thead>
<tr>
<th>Form</th>
<th>Subunit composition</th>
<th>Processivity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holoenzyme</td>
<td>(\alpha, \epsilon, \theta, \tau, \gamma, \delta, \delta', \chi, \psi, \beta)</td>
<td>&gt;50,000, 150,000</td>
<td>38, 39</td>
</tr>
<tr>
<td>Reconstituted holoenzyme minus (\tau)</td>
<td>(\alpha, \gamma, \delta, \delta', \chi, \psi, \beta)</td>
<td>1–3,000</td>
<td>40</td>
</tr>
<tr>
<td>Pol III*</td>
<td>(\alpha, \epsilon, \theta, \psi)</td>
<td>200</td>
<td>41</td>
</tr>
<tr>
<td>Pol III</td>
<td>(\alpha, \epsilon, \theta, \tau)</td>
<td>60</td>
<td>41</td>
</tr>
<tr>
<td>Pol III (core)</td>
<td>(\alpha, \epsilon, \theta)</td>
<td>10</td>
<td>38</td>
</tr>
</tbody>
</table>
metric dimer hypothesis. $\tau$ and $\gamma$ appear to compete for the same binding sites within holoenzyme, suggesting a similar role for the two proteins (53). Both $\tau$ and $\gamma$ are present in every active holoenzyme molecule (59). This eliminates the possibility that $\tau$ and $\gamma$ randomly assort during holoenzyme assembly in vivo as they apparently do in vitro. The mechanism that operates to ensure an asymmetric structure in vivo is unclear. Perhaps the holoenzyme assembles in the cell at the origin of replication where asymmetric protein-protein interactions at the initiating replication fork direct asymmetric assembly. Consistent with such a mechanism, genetic evidence has been obtained for an interaction between the origin-specific dnaA gene product and a dnaX product, either $\gamma$ and/or $\tau$ (60).

The $\tau$ and $\gamma$ subunits appear to contain the site that sets the ATP-dependent clamp on primed DNA. A consensus ATP binding sequence is located within the amino-terminal sequences common to both proteins (19). Both $\tau$ and $\gamma$ bind ATP with a dissociation constant of $\sim 2 \, \mu M$ (61). $\tau$, but not $\gamma$, is a DNA-dependent ATPase by itself (61, 62).

$\tau$ and $\gamma$ have differential interactions with replication proteins. $\gamma$ is isolated in a complex with $\delta$, $\delta'$, $\psi$, and $\chi$ (29, 30). $\tau$ has only been isolated as a stable complex with pol III or by itself. In a technically difficult and elegant study, O'Donnell and Studwell (31) resolved the $\gamma\delta$ complex to yield $\delta$ and $\delta'$. They demonstrated that $\delta$ stimulates $\gamma$ and $\tau$, while $\delta'$ only stimulates $\tau$ in formation of preinitiation complexes in the presence of $\beta$.

The above information suggests related but discrete roles for the $\gamma$ and $\tau$ subunits. This is consistent with the original hypothesis of an asymmetric dimer and supports the notion that an asymmetric placement of the $\tau$ and $\gamma$ subunits within the replicative complex determines both structural and functional asymmetry. Among the critical experiments required to test the unity of these two hypotheses are (i) a determination of whether either $\gamma$ or $\tau$ complexes uniquely use ATP$\gamma$S to form initiation complexes and (ii) a direct determination of the subunit arrangements within the pol III holoenzyme.

If the holoenzyme does exist as an asymmetric dimer, how does it function to rapidly recycle on the lagging strand of the replication fork? Without excess auxiliary subunits, holoenzyme cycles very slowly. Using different preparations of holoenzyme and differing reaction conditions, times from 30 min to 1–2 min have been estimated (42, 44, 63). Although quantitatively different and largely dependent upon the ratio of excess auxiliary subunits present, they are drastically longer than the value of under 1 s required in vivo. O'Donnell and colleagues (31, 63) have demonstrated that formation of a preinitiation complex can markedly facilitate the cycling of polymerase core from a completed initiation complex to the next primer. In a corollary of the asymmetric dimer hypothesis, they have proposed that this mechanism might be exploited in vitro to facilitate rapid cycling by a polymerase that contains two $\gamma\delta$ complexes in the lagging strand half and a $\tau\delta'$ complex in the leading strand half (31, 63, 64). The two $\gamma\delta$ complexes within the holoenzyme could alternate between completed Okazaki fragments and the next primer. Whether the stoichiometry of these components within the holoenzyme supports this possibility is not yet certain. Although formation of a preinitiation complex bypasses a rate-limiting step in initiation complex formation, questions regarding the availability of $\gamma\delta$ complex to enable such a reaction remain. For the first $\gamma\delta$ complex used in the synthesis of two Okazaki fragments to be reused for a third fragment in a series along the lagging strand of the replication fork requires for it to be released from the terminus of the first fragment. The release of $\gamma\delta$ complex from products appears to be very slow relative to the required rates of polymerase cycling (31, 63). Thus, the cycling problem is just passed back one step and becomes related to the question originally stated at the holoenzyme level. How is $\gamma\delta$ complex released and recycled at a rate of 1/s so that it is available for the next Okazaki fragment synthesized? The answer may be found in special interactions found within the holoenzyme.

Another special feature of the holoenzyme, initially thought to be involved in polymerase cycling, is its ability to jump over primers (65, 66). Holoenzyme can very rapidly transfer intramolecularly between primers, provided they are ahead of the advancing polymerase (66). Since this direction is the opposite of that required for lagging strand cycling, it is probably not involved in cycling, but instead in jumping over annealed oligonucleotides at the replication fork that would otherwise stall polymerase movement. Consistent with this notion, $\tau$, the subunit proposed to be associated with the leading strand half of holoenzyme, is apparently required for primer jumping (67).

**Structure of the DNA Polymerase III Holoenzyme**

Central to the understanding of cycling, asymmetric dimers, and related issues is the determination of the structure of the pol III holoenzyme at the level of subunit arrangements and subunit-primer-template contact. One could expect protein-protein cross-linking, site-specific protein-DNA cross-linking, fluorescence energy transfer between labeled subunits and sites, electron microscopy, and eventually, x-ray crystallography all to make contributions to this important endeavor.

Presently, our understanding of the structure of the holoenzyme is limited to information gained through functional interactions detected and suppressor mutations. A preliminary model for holoenzyme subunit-subunit interactions is shown in Fig. 2. $\alpha$ and $\epsilon$ form an isolable complex upon mixing (68). Mutations in the structural gene for $\epsilon$ have also been found that suppress dnaE ($\alpha$) mutations (69). The assumption usually made is that suppressor mutations arise through modification of a subunit that interacts directly with the suppressed mutant gene product. pol III core (exec) is isolable, but we don't know with which subunit(s) $\delta$ interacts (13). $\tau$ can be isolated in a complex with pol III core; the contacted subunit is unknown (18). Suppressor data suggest an interaction between $\gamma$ and/or $\tau$ and $\beta$ (70). Either $\gamma\delta$ or $\tau$ in the presence of either $\delta$ or $\delta'$ can transfer $\beta$ to primed DNA to form a preinitiation complex (31). Suppressor data indicate an interaction between $\beta$ and $\alpha$ (71). Reinforcing this conclusion, we also know that $\beta$ can interact with and increase the processivity of core pol III (72). Genetic evidence for $\alpha$-$\alpha$
interaction and pol III holoenzyme being a dimer was obtained through dnaE interallelic complementation (73). From biochemical studies, it is known that \(\delta\) can interact with \(\gamma\) and either \(\delta\) or \(\delta'\) with \(\tau\). \(\chi\) and \(\psi\) have been isolated in a complex with \(\gamma\) (31).

My laboratory has taken a cross-linking approach to identify specific subunit-subunit and subunit-primer-template contacts and a parallel approach using fluorescence energy transfer to identify distances between sites within replication complexes. In initial studies using fluorescence energy transfer, we have focused on the \(\beta\) subunit, \(\beta\) exists as a dimer free in solution by itself. Addition of Mg\(^{2+}\) at physiological concentrations triggers dissociation and an accompanying conformational change in the vicinity of Cys\(_{333}\) on the distal ends of the \(\beta\) dimer (74, 75). Within the initiation complex, \(\beta\) exists in a ratio of 1:1 with primer, with Cys\(_{333}\) located ~65 Å from the antipernucleotide nucleotide of the primer.\(^5\) Continuation of these studies should permit us to assign the subunit arrangement within holoenzyme. This knowledge coupled with that gained through sophisticated functional studies being conducted in several laboratories should lead to an understanding of the individual roles of subunits within the complex replicative apparatus and the cooperation that occurs between components enabling a coordinated synthetic reaction to occur at the replication fork.

Note Added in Proof—Recent work directed toward assigning the two genes for the last five holoenzyme components has revealed two genes that have already been identified as open reading frames.\(^6\) The gene for \(\delta\) is located immediately downstream of \(\rho\) at 16 min (76), and the structural gene for \(\psi\) is located between \(\alpha\) and \(\psi\) at 97 min (77). This work has also revealed that the structural gene for \(\beta'\) is distinct from \(\beta\).

REFERENCES

