Exonucleolytic proofreading in DNA replication

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USyd Chem 24-04-07
BIDIRECTIONAL SEMIDISCONTINUOUS REPLICATION FOLLOWING INITIATION AT oriC

Diagram showing the replication process with forks, leading and lagging strands, and the oriC region.
The *E. coli* replication fork
The *E. coli* replisome

Schaeffer (2005) *IUBMB Life*
The asymmetric dimer of DNA polymerase III holoenzyme
**DNA POLYMERASE III HOLOENZYME**

**HOLOENZYME PROPERTIES**
- Efficiency (~1000 Nt/sec)
- Processivity (>5000 Nt)
- Fidelity (1:10^{7}-10^{8})

<table>
<thead>
<tr>
<th>subassemblies</th>
<th>subunit</th>
<th>size (kDa)</th>
<th>gene</th>
<th>functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>pol III’</td>
<td>α</td>
<td>132</td>
<td>dnaE</td>
<td>polymerase 5’→3’</td>
</tr>
<tr>
<td></td>
<td>ε</td>
<td>27</td>
<td>dnaQ</td>
<td>proofreading exonuclease 3’→5’</td>
</tr>
<tr>
<td></td>
<td>θ</td>
<td>10</td>
<td>hoIE</td>
<td>binds ε, stabilizes core</td>
</tr>
<tr>
<td></td>
<td>τ</td>
<td>71</td>
<td>dnaZX</td>
<td>dimerization of core</td>
</tr>
<tr>
<td>pol III*</td>
<td>γ</td>
<td>52</td>
<td>dnaZX</td>
<td>ATP-dependent β clamp loader</td>
</tr>
<tr>
<td></td>
<td>δ</td>
<td>35</td>
<td>hoIA</td>
<td>β clamp loader</td>
</tr>
<tr>
<td></td>
<td>δ’</td>
<td>33</td>
<td>hoIB</td>
<td>β clamp loader</td>
</tr>
<tr>
<td>γ complex</td>
<td>χ</td>
<td>15</td>
<td>hoIC</td>
<td>β clamp loader; SSB interaction</td>
</tr>
<tr>
<td></td>
<td>ψ</td>
<td>12</td>
<td>hoID</td>
<td>β clamp loader (?)</td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>37</td>
<td>dnaN</td>
<td>sliding clamp (processivity)</td>
</tr>
</tbody>
</table>
Families of DNA polymerases

Pol A: DNA polymerase I (Pol I)
   Klenow; Taq Pol; T7 Pol

Pol B: DNA polymerase II (Pol II)
   T4 Pol; RB69 Pol; Pol α

Pol C: DNA polymerase III (Pol III)

repair polymerases (families X and Y)
The core of DNA polymerase III

\[ \alpha \ 5'\text{-}3' \text{ polymerase} \]
\[ \epsilon \ 3'\text{-}5' \text{ proofreading exonuclease} \]
\[ \theta \text{ stabilizes } \epsilon \]

\[ \alpha \text{ interacts with } \epsilon \text{ (} \alpha.\epsilon \text{ complex)} \]
\[ \epsilon \text{ interacts with } \theta \text{ (} \epsilon.\theta \text{ complex)} \]
\[ \text{no interaction between } \alpha \text{ and } \theta \]
Structure of the $\alpha$ subunit

Lamers et al. (2006) Cell 126, 881–892
Bailey et al. (2006) Cell 126, 893–904
Structure of the $\alpha$ subunit

Lamers et al. (2006) Cell 126, 881–892
Bailey et al. (2006) Cell 126, 893–904
The $\varepsilon$ subunit is the replicative proofreader ($3'\rightarrow 5'$ exonuclease)

DNA substrate
(mismatched primer-template)

5'-$p$-nitrophenyl ester of thymidine-5’-phosphate ($p$NP-TMP)
Domain structure of ε

Hamdan et al. (2000)
J. Struct. Biol., 131, 164-169
Overproduction, assay and purification of ε186

Hamdan et al. (2002) Biochemistry, 41, 5266-5275
ε186 bears the active site of ε & the ε186.θ complex

<table>
<thead>
<tr>
<th></th>
<th>$K_M$ (mM)</th>
<th>$k_{cat}$ (min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε</td>
<td>0.95</td>
<td>148</td>
</tr>
<tr>
<td>ε186</td>
<td>1.08</td>
<td>293</td>
</tr>
<tr>
<td>ε186.θ</td>
<td>1.51</td>
<td>215</td>
</tr>
</tbody>
</table>

Hamdan et al. (2002)  
*Biochemistry, 41, 5266-5275*
Hydrolysis of \( p \text{NP-TMP} \) by \( \varepsilon 186 \) requires divalent metal ions.  

<table>
<thead>
<tr>
<th></th>
<th>( K_{Me} ) (mM)</th>
<th>( k_{cat} ) (min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn(II)</td>
<td>0.31</td>
<td>334</td>
</tr>
<tr>
<td>Mg(II)</td>
<td>6.9</td>
<td>19.9</td>
</tr>
</tbody>
</table>

Hamdan et al. (2002)  
*Biochemistry*, 41, 5266-5275
Hydrolysis of $\rho$NP-TMP by $\epsilon$186 is competitively inhibited by dNMPs

Hamdan et al. (2002) Biochemistry, 41, 5266-5275; S. Hamdan, unpublished
**ε186 structure determination**

- Native and Se-Met protein crystals
  - 0.1 mM cacodylate, pH 5.8, 21% PEG-8K
  - 2.5 mM TMP, 2.5 mM Mn$^{2+}$

- X-Ray diffraction (synchrotron sources)
  - Native crystal at pH 5.8 - 1.7 Å
  - Native crystal at pH 8.5 - 1.8 Å
  - Se-Met - 1.8 Å
  - Crystals isomorphous:
    - (P4$_1$2$_1$2$_1$; a = b = 60.8 Å, c = 111.1 Å)

- MAD experiment at 3 wavelengths on Se-Met edge

- Native structures refined using Se-Met model
  - Native (pH 5.8) $R/R_{\text{free}} = 0.201/0.234$
  - Native (pH 8.5) $R/R_{\text{free}} = 0.199/0.227$

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**Hamdan et al. (2002) Structure 10, 535-546**
Crystal structure of \( \varepsilon \text{186-Mn}_2\text{-TMP} \)
Structure of ε186-Mn₂-TMP

Hamdan et al. (2002)
Structure 10. 535-546
DNA polymerase proofreading domains

A

\(\varepsilon_{186}\)

B

DNA polymerase I

C

T4 DNA polymerase

D

T7 DNA polymerase

E

RB69 DNA polymerase

F

*Thermococcus gorgonii*
DNA polymerase

G

Archaeon D.Tok
DNA polymerase

H

Archaeon Sso
DNA polymerase
*Sulfolobus solfataricus*

I

Archaeon Kod 1
DNA polymerase
*Pyrococcus kodakaraensis*
Single-domain 3’-5’ exonucleases

A

\[ \epsilon_{186} \]

B

Exonuclease I

C

Human 3’hExo

D

Human ISG20

E

RNase D

F

Yeast Pop2

G

Human Trex2

H

Haemophilus influenzae Oligoribonuclease
ε186 and the 3’-5’ exo domain of *E. coli* exonuclease I

Active site of ε186-Mn$_2$-TMP
Active sites of 3’-5’ exonucleases

Hamdan et al. (2002) Structure 10, 535-546
pH-dependent changes in TMP conformation at the active site of ε

pH-dependence of the structure of the active site of ε
Hypothetical water exchange reaction at the active site of $\varepsilon$. 

At high pH ($\varepsilon$ 186):
- **His162**
- **Asp167**
- **Asp12**
- **Glu14**

At low pH ($\varepsilon$ 186):
- **Asp103**
- **Asp167**
- **Glu14**
- **Asp12**

The diagram illustrates the exchange process between points A and B.
pH Rate Profile (pNP-TMP hydrolysis)

\[ K_{m1} = K_{m2}, \text{ so } pK_{EH} = pK_{ESH} \]

\[ k_1 = 50 \text{ min}^{-1}; \ k_2 = 400 \text{ min}^{-1} \]

Hamdan et al. (2002) Biochemistry, 41, 5266-5275
Mechanism of pNP-TMP hydrolysis at the 3’-5’ exonuclease site of ε at high pH

Hamdan et al. (2002)
Biochemistry, 41, 5266-5275
Possible mechanism of \( p\)-NP-TMP hydrolysis at the 3'-5' exonuclease site of \( \varepsilon \) at low pH?
Mechanism of DNA hydrolysis at the 3’-5’ exonuclease site of ε

Hamdan et al. (2002)
*Structure* 10. 535-546
A second disordered TMP molecule near the active site of ε186

Model of $\varepsilon_{186}$-DNA complex

Experimental Pol I exo-DNA  

Model $\varepsilon$-DNA
A model for the interaction of primer-template DNA with ε186