interpreted as a demonstration of a biologically significant protein–protein interaction. The two proteins must be shown to be involved in the same process in vivo; ideally the interaction should be demonstrated by a functional assay or by any of several genetic techniques that can detect protein–protein interactions. Nevertheless, protein affinity chromatography is a useful technique, both as an initial method for detecting the potential components of protein machines and for the purification of such components. Protein affinity chromatography provides a unique opportunity to exploit the forces that cause protein machines to assemble to allow exploration of the composition and regulation of these complexes that act in so many cellular processes.

[32] Radiolabeling of Proteins for Biochemical Studies

By Zvi Kelman, Vytautas Naktinis, and Mike O'Donnell

Introduction

Several processes in nucleic acid metabolism such as replication, transcription, and translation require the coordinated action of numerous proteins. This coordination is manifest through protein–protein and protein–nucleic acid interactions ranging from stable complexes to transient contacts. Important questions in these multicomponent systems include the following: How much of each protein is present in protein complexes or on the nucleic acid? Is the protein interacting with a specific nucleic acid structure or sequence? How tightly associated are the subunits of a complex, and how are these interactions influenced by other proteins or by ATP? Which surfaces on a protein interact with other proteins? Answers to questions such as these require a convergence of several experimental techniques. Some techniques that address these subjects utilize radioactive proteins.

This chapter describes two radioactive labeling methods and illustrates a few applications of labeled proteins in biochemical studies. One method is reductive methylation,\textsuperscript{1-3} which was described in this series using $[^{14}\text{C}]$formaldehyde.\textsuperscript{2} We present a modified protocol using NaB$_3$H$_4$ that

\begin{enumerate}
\item G. E. Means, this series, Vol. 47, p. 469.
\item G. E. Means and R. E. Feeney, Biochemistry \textbf{7}, 2192 (1968).
\end{enumerate}
introduces only 1 or 2 [3H]methyl groups per protein for a specific activity of 20 to 40 Ci/mmol (0.5 to 1.0 × 10⁶ cpm/μg of 50-kDa protein). The second method uses a specific protein kinase to 32P-end-label a protein into which a 5–7 amino acid residue kinase recognition motif has been engineered onto the N or C terminus. This procedure results in a specific activity up to 3000 Ci/mmol (>60 × 10⁶ cpm/μg of 50-kDa protein). We have no experience in performing protein labeling using isotopes of iodine and for this the reader is referred to an excellent treatise in this series.

A drawback to chemical labeling is that the modified protein may lose one or more of its activities. Hence, the labeled protein must be tested for activity relative to unlabeled protein. Reductive methylation is the least invasive technique because it introduces only one or two methyl groups. In our experience, 12 of the 13 different proteins that were labeled retained 85 to 100% activity. End-labeling with 32P introduces a larger modification and some proteins may be expected to lose function, but the three proteins we have 32P-end-labeled retain their activity.

Proteins that are radiolabeled metabolically (in vivo) with either [35S]methionine or 3H- or 14C-labeled amino acids should retain full activity. However, the drawback to metabolic labeling is the need to purify the radioactive protein, and during the prolonged use of centrifuges and cold room equipment it is difficult to contain the radioactivity. The chemical labeling methods described here also carry a health hazard, but the procedures can be performed in a fume hood in a matter of a few hours. It is important that the operations be performed using all available precautions including gloves, laboratory coat, and film badge, and that proper guidelines are followed to dispose of the radioactive waste.

3H-Labeling by Reductive Methylation

The procedure described here modifies only a few lysine residues per protein molecule and is adapted from methods used in Arthur Kornberg’s laboratory. The reaction, outlined in Eq. (1), involves the addition of formaldehyde, which forms a Schiff base with lysine followed by reduction using NaB₃H₄ and results in net replacement of a proton for a [3H]methyl group on the primary amine of lysine. Once lysine is methylated, it is more reactive toward a second round of methylation. The charge of the methylated lysine is conserved; the pK of monomethylated lysine is in-

---

6 C. W. Parker, this series, Vol. 182, p. 721.
creased by about 0.3 pH unit and the pK of dialkylated lysine is lower than lysine by about 0.6 pH units. When only a few methyl groups are incorporated into a protein, the monoalkylated lysine species predominates.

\[
\text{Protein-NH}_2 + C \rightarrow O \rightarrow \text{Protein-N=C} \rightarrow \text{Protein-N-C}^3\text{H} (1)
\]

The extent and velocity of the reaction increases with pH, very little reaction occurs at pH 7.0 and maximal labeling is achieved at pH 9.5. The elevated pH is needed to form the Schiff base between lysine and formaldehyde (a readily reversible reaction), and to preserve the sodium borohydride, which is rapidly decomposed as the pH is lowered. To prevent extensive labeling the reaction is carried out at pH 8.5. The reaction can be performed at pH 7.0 using cyanoborohydride, although the temperature must be elevated and the reaction time may be longer. Reductive methylation occurs only with lysine and with the amino terminus, no reaction occurs with side chains other than lysine, and disulfide bonds are not reduced. Most of the radioactivity is not incorporated into protein but is consumed by side reactions such as reduction of formaldehyde to methanol and breakdown of borohydride by solvent. Buffers containing primary and secondary amines must be avoided because they form the Schiff base and consume the reactants. We have used sodium borate although buffers with tertiary amines or phosphate buffer can be used. Besides formaldehyde, other aldehydes and ketones can be used (e.g., acetone and acetaldehyde), but the resulting alkyl group on the lysine is then bulkier (isopropyl and ethyl, respectively). Hence we use formaldehyde to produce the smallest modification possible. Note that formaldehyde itself can modify proteins by forming inter- and intraprotein cross-links; however, the destructive reactions of formaldehyde with proteins during reductive alkylation has been examined with none of these products being observed under conditions that are much more extensive in time and temperature than those used here.

**Materials for Reductive Methylation**

We usually purchase 0.5 Ci of NaB\(^3\)H\(_4\) of 50 to 75 Ci/mmol (Du Pont–New England Nuclear, NET-023X). 0.5 Ci of 75 Ci/mmol NaB\(^3\)H\(_4\) is 6.7 \(\mu\)mol, which is sufficient to label 3.3 ml of protein. We usually label 1 ml

---

of 3 to 5 different proteins on the same occasion. Unfortunately, NaB\textsuperscript{3}H\textsubscript{4} of this specific activity is not available in smaller quantities. Lower specific activity material can be purchased such as 100 mCi NaB\textsuperscript{3}H\textsubscript{4} at 5 to 15 Ci/mmol (NET-023H). Because most of the radioactivity is lost in competing side reactions, the higher the concentration of the protein, the more labeled protein is produced per mole of NaB\textsuperscript{3}H\textsubscript{4} (i.e., the resulting specific activity of the protein is unaffected whether a 1 or a 5 mg/ml solution of protein is used). Protein can also be labeled using \[^{14}\text{C}]\text{formaldehyde,}\textsuperscript{3} but the specific activity is 1000-fold lower than NaB\textsuperscript{3}H\textsubscript{4}.

The other necessary materials include a small container for radioactive solid waste, a triangular file, 1 to 200-\textmu l Pipetman with an extended tip (explained below), ice bucket with each dialyzed protein in a separate open tube, 10 mM NaOH on ice, 2 M formaldehyde on ice, 1 M lysine, and an empty Eppendorf tube (for the NaB\textsuperscript{3}H\textsubscript{4} after it is dissolved in 10 mM NaOH). To separate protein from reagents after the reaction, one fraction collector is needed for each protein, 10 ml columns of Sephadex G-25 packed in disposable 10-ml plastic pipettes with a glass wool plug, and gel filtration column buffer. Our typical column buffer is 20 mM Tris-HCl, pH 7.5, 0.5 mM EDTA, 2 mM dithiothreitol (DTT), and 20% glycerol.

**Procedure for Reductive Methylation**

The night before the labeling reaction, dialyze each protein at a concentration of 1 to 5 mg/ml into 50 mM sodium borate, pH 8.5, 0.5 mM EDTA, 10% glycerol (DTT can be included). The next day the reagents and hardware must first be arranged in the fume hood. Then the sealed ampule containing the dry NaB\textsuperscript{3}H\textsubscript{4} is gently tapped to place all the powder at the bottom of the vial and the file is used to score the neck of the ampule. The ampule will have \textsuperscript{3}H\textsubscript{2} gas under pressure and it is important when snapping the neck of the ampule to position it near the hood exhaust port. Dissolve the NaB\textsuperscript{3}H\textsubscript{4} powder in 10 mM NaOH for a final concentration of 100 mM NaB\textsuperscript{3}H\textsubscript{4} (this will be in the range of 67 to 100 \textmu l depending on the specific activity of the NaB\textsuperscript{3}H\textsubscript{4}). Due to the length of the ampule and the narrow opening at the neck, the Eppendorf tip must be modified so that it reaches to the bottom of the ampule. A simple modification is to fasten a 2-in. length of polyethylene tubing onto the tip. The 10 mM NaOH is drawn into the Pipetman and then transferred into the ampule to dissolve the NaB\textsuperscript{3}H\textsubscript{4}, then withdrawn and placed into the empty Eppendorf tube on ice. At this point one must work quickly, but steadily, because the reagent is decomposing (as evidenced by the slow appearance of bubbles).

The labeling reaction is initiated upon adding formaldehyde to the protein(s) to a final concentration of 20 mM and then NaB\textsuperscript{3}H\textsubscript{4} to a final
concentration of 2 mM. After a further incubation of 15 min on ice, stop the reaction by adding lysine to a final concentration of 50 mM, and separate the protein from the reagents by passing the reaction mixture over a 10-ml column of Sephadex G-25. The column is disposed of as radioactive waste at the end of the procedure. The column is initially prepared in the cold room and the buffer is kept on ice, but just before use it is brought into the fume hood along with a fraction collector. Fractions of 400 µl are collected and placed on ice. A small amount (3 µl) of each fraction is counted to locate the protein peak, these are pooled, the protein concentration is determined, and then the [3H]protein is aliquoted and stored frozen at -70°C. The labeling procedure should take no more than 15 to 20 min, the gel filtration about 30 min, and the counting and pooling of column fractions, protein determination, and aliquoting may take about 1 hr.

Variables in this reaction include time, pH, temperature, and concentrations of formaldehyde and NaB³H₄. We find that under the conditions described above, the rate of methylation is nearly linear for 10 min and levels off by 15 min. Also, lowering the formaldehyde from 20 to 10 mM yields half the level of methylation. Finally, use of 2 mM NaB³H₄ is sufficient for the low level of labeling desired; 4 mM NaB³H₄ increased the extent of labeling by only 20%, and thus is put to better use at 2 mM to label more protein. We have always performed these procedures on ice and have not experimented with different temperatures. We have examined the effect of increasing the pH to 9.0 and find the extent of methylation increases at least 1.5-fold, but pH 8.5 is less harsh on the protein and is sufficient for incorporation of one to two methyl groups.

³²P-End-Labeling of Proteins

An efficient substrate for the cAMP-dependent protein kinase has been identified as a heptapeptide Leu-Arg-Arg-Ala-Ser-Leu (or Val)-Gly (or Ala). The Kₘ of this sequence for phosphate transfer to the Ser by the kinase is 10 to 20 µM. Use of only the five inner residues results in an eightfold increase in Kₘ, and substitution of any of the five inner residues increases the Kₘ by 10-400 fold, resulting in low to negligible rates of phosphorylation. Radioactive proteins of high specific activity (>3000 Ci/mmol) have been produced upon cloning five to seven residues of this sequence onto the C terminus of human interferon α and onto the N terminus of segments of c-Fos.

We have engineered the kinase recognition sequence onto either the

---

A) N-terminus

5' - CTGGCGCATATGCTTCGAAGAGCTTTGTT-[15 matching nucleotides] - 3'

NdeI site BstBI site

B) C-terminus

3' - [15 matching nucleotides]-GAAGCTTCTCGAAGACAACCAATTCCTAGGTGGTC - 5'

BstBI site BamHI site

**Fig. 1.** Oligonucleotide sequences for PCR amplification of either N- or C-terminal PK protein. The oligonucleotides shown are designed for use with a second oligonucleotide that matches the gene of interest to produce a PCR product encoding either (A) an N-terminal PK protein or (B) a C-terminal PK protein. The encoded amino acids of the kinase recognition motif are shown above the nucleotide sequence. In each case, the 15 nucleotides at the 3' terminus match the gene of interest. The NdeI and BamHI cloning sites and the BstBI screening site are marked. The 5-6 nucleotides at the 5' terminus of these oligonucleotides ensure efficient cleavage of the PCR product.

N or C terminus of three proteins, expressed them in *E. coli*, and purified them to homogeneity. We have mainly studied the β subunit of *E. coli* DNA polymerase III holoenzyme (Pol III) in which a six-residue site, Leu-Arg-Arg-Ala-Ser-Val (followed by Pro), was engineered onto the C terminus. In addition, we have placed the seven-residue site, Leu-Arg-Arg-Ala-Ser-Val-Gly, onto the C terminus of the λ cro repressor and of EBNA1, the latent origin binding protein of the Epstein-Barr virus. We have also placed the seven-residue sequence Met-Leu-Arg-Arg-Ala-Ser-Val onto the N terminus of β and cro. These protein kinase (PK) proteins were engineered by PCR using an oligonucleotide encoding the kinase motif (Fig. 1) and expressed in the pET-3 system. The kinase recognition motif can also be introduced by site-directed mutagenesis. Since the pET-3 system was used for expression, the primer sequences contained an NdeI site for N-terminal PK proteins, and a BamHI site for C-terminal PK proteins. The BstBI site is not present in pET-3 and thus aids in screening clones.

**Procedure for 32P-End-Labeling**

Unlike reductive methylation, this technique can be performed with small amounts of protein and the procedure is compatible with a variety

---

of buffers. We typically use a volume of 30 to 120 μl containing 0.2 to 6 nmol PK protein in 20 mM Tris–HCl, pH 7.5, 1 to 60 μM [γ-32P]ATP (specific activity discussed below), 2 mM DTT, 100 mM NaCl, 12 mM MgCl2, and 10 mM NaF. Labeling is initiated upon adding cAMP-dependent protein kinase (0.008 U kinase/nmol PK protein) and shifted to 37°. After 10 min at 37°, the reaction is stopped on addition of 30 mM EDTA (final concentration). The extent of 32P incorporated into the PK protein can be determined by acid precipitation or by analysis in a SDS–polyacrylamide gel (for the latter, addition of 5 mM unlabeled ATP to the sample buffer lowers background radiation). Free [γ-32P]ATP can be removed either by ultrafiltration, spin dialysis, or gel filtration. The catalytic subunit of cAMP-dependent protein kinase from bovine heart can be purchased from Sigma and was used in previous studies, however, we have used the murine version expressed and purified from E. coli (gift of Dr. Susan S. Taylor, University of California at San Diego).

Under these conditions the stoichiometry of P1 incorporation is typically 0.5 to 0.8 mol P1/mole protein (except N-terminal labeled βPK, discussed below). Before embarking on these studies, it is important to determine whether the wild-type protein is phosphorylated by protein kinase. Wild-type β was not phosphorylated to a significant extent (<0.9 mmol P1/mol βPK) and sequence analysis of the radioactive chymotryptic peptide of [32P]βPK confirmed the label was in the kinase motif at the C terminus.

High specific activity protein can be achieved using straight [γ-32P]ATP (3000 Ci/mmol) at approximately 1 μM, or the relative proportion of unlabeled ATP and radioactive ATP can be adjusted to achieve the desired specific activity. This can be very important in experimental designs using both 3H-labeled protein and 32P-labeled protein in the same experiment because the specific activity of 3H-labeled proteins is only 20 to 40 Ci/mmol and therefore one must take care to prepare the 32P-labeled protein with a comparable specific activity so as not to encounter problems of bleed over between windows while counting both isotopes.

**Important Considerations**

One advantage to the 32P-end-labeling method is that the isotope is available in small quantities and the manipulations are similar to end-labeling DNA, a common laboratory technique. PK proteins can also be labeled using [γ-35S]ATP. How often do labeled PK proteins retain activity? The modification is

substantial; not only are several amino acids added to the protein, but a large phosphate group is also present. In our experience the activity of both N- and C-labeled $[^{32}\text{P}]\beta\text{PK}$ is unchanged in several assays including assembly of $[^{32}\text{P}]\beta\text{PK}$ onto primed DNA by Pol III, and ability of $[^{32}\text{P}]\beta\text{PK}$ to confer highly processive DNA synthesis onto the polymerase. Likewise, the $[^{32}\text{P}]\text{EBNA1PK}$ and both N- and C-labeled $[^{32}\text{P}]\text{croPK}$ retained their site-specific DNA binding activities. It is important to note that if the amount of ATP used in the labeling reaction is substoichiometric to the protein (often the case in DNA end-labeling reactions), some types of activity assays will not truly evaluate whether the phosphorylated species retains activity.

In theory one should be able to place the kinase motif at any exposed region of a molecule and label it. However, placement of this kinase motif at other positions in the $\beta$ subunit has revealed limitations to this technique. For example, the N-terminal $\beta\text{PK}$ is labeled approximately 4% at best. The problem is likely due to the inability of the kinase to gain access to the recognition site as addition of 0.5 M urea increased the labeling to 16%. Further, we have placed the seven-residue kinase recognition sequence into two different internal positions of $\beta$, which are located on the surface according to the crystal structure, but the kinase did not phosphorylate these proteins.

Examples of Applications

*Interaction of Proteins with DNA*

To identify a particular subunit on DNA in a complex mixture of several proteins, the reaction is analyzed by gel filtration using beads with large pores such that free proteins elute in the included fractions and resolve from proteins bound to the large DNA (e.g., plasmid or M13 ssDNA), which elute in the excluded fractions. For these studies we have used agarose beads Bio-Gel A-5m and A-15m (Bio-Rad) as well as Sepharose 4B (Pharmacia-LKB), which exclude plasmids and ssDNA phage genomes, yet include proteins of up to 1 MDa.

Examples of this technique abound in studies from Arthur Kornberg's laboratory. For example, $^3\text{H}$-labeled proteins were used to identify primosomal proteins that remained on the $\phi X174$ ssDNA with the finding that the DnaC protein, while essential to assemble the primosome on DNA, does not remain on the DNA. The *E. coli* Pol III contains a five-subunit

---

Fig. 2. Applications of radiolabeled proteins to study the molecular dynamics and structural details of multicomponent complexes. (A) The [\( ^{3}H \)]y complex matchmaker assembles the [\( ^{32}P \)]βPK dimer onto SSB coated primed M13mp18 ssDNA. Gel filtration on Bio-Gel A-5m resolves the [\( ^{32}P \)]βPK bound to DNA (fractions 10–15) from the [\( ^{3}H \)]y complex that dissociates from DNA and elutes in the included fractions. The experiment was performed essentially as described in Fig. 2.3 of Stukenberg.\(^{14}\) (B) During gel filtration, [\( ^{3}H \)]β clamps coelute with
subassembly called the γ complex, which acts in a similar fashion as DnaC protein; γ complex places the β subunit of Pol III onto a primed template but then departs from the DNA. Figure 2A shows the use of two labeled subunits in this latter reaction. The ³H-labeled γ complex was used to place[^32P]βPK onto a primed template and the reaction was analyzed by gel filtration. Analysis of the column fractions shows a stoichiometric amount of[^32P]βPK dimers comigrating with the DNA, but the [³H]γ complex migrates as free protein complex in the included fractions.

Topological Binding of Protein to DNA

The β subunit of Pol III is a ring-shaped dimer that completely encircles DNA. Once the β ring is assembled onto DNA (by the γ complex and ATP) it tethers the rest of the Pol III machinery to the template and slides along with it for highly processive DNA synthesis. This simple solution

to high processivity may generalize to the gene 45 protein processivity factor of the phage T4 replicase, and the PCNA processivity factor of the yeast and human polymerase δ. Protein surrounding DNA seems so basic that it may even generalize to other DNA metabolic machineries. For example, replicative helicases such as those encoded by phage T4 and phage T7, E. coli DnaB, and simian virus 40 (SV40) T antigen are all hexamers and may surround duplex DNA for their action (i.e., like the sixfold pseudosymmetric β dimer).

Initial experiments using [3H]β revealed this “topological binding” mode of β to DNA and predicted its ring shape, thus motivating the crystal structure analysis. A particularly telling experiment is shown in Fig. 2B. Here, [3H]β was placed onto a singly nicked plasmid DNA (by γ complex and ATP) and then gel filtered to reveal that several β dimers coelute with the DNA in the excluded fractions. However, linearization of the DNA results in dissociation of β, implying that it slides along DNA and falls off the end. If β were to bind DNA by direct chemical interaction, as other DNA binding proteins do, then it would have stayed associated with the linear DNA through gel filtration as well as with circular DNA. Hence, β must be bound physically to DNA by virtue of its topology. This type of experiment is rather simple and variations on this theme may help identify the topological binding proteins of other systems.

Subunit Exchange in Multiprotein Complexes

Radiolabeled protein can be used to measure the rate of dissociation of a subunit from within a multiprotein complex. As an example, in Fig. 2C the five-subunit γ complex (γδδ′χψ) was labeled by reconstituting it using [3H]δ′. To this was added an eightfold molar excess of unlabeled δ′ and the mixture was gel filtered at various times. Whenever the [3H]δ′ subunit dissociates from the complex, an unlabeled δ′ takes its place. Hence, over time, the column fractions containing γ complex decrease in radioactivity and column fractions containing free δ′ increase in radioactivity. This technique can be extended to determine how the rate of subunit exchange is influenced by DNA, nucleotides and other proteins.

Weak Protein–Protein Interactions

Only strong interactions among proteins can be detected by gel filtration because it is not an equilibrium technique. However, weak interactions can be quantitated using the equilibrium gel filtration technique. This technique is normally used to determine the $K_d$ between a protein and a

---

small ligand molecule (e.g., a nucleoside triphosphate) where the ligand is radiolabeled and is present throughout the column buffer. The protein binds the radiolabeled ligand and then elutes ahead of the ligand, resulting in a peak of radioactivity that emerges above the baseline level and is followed by a trough where the unbound ligand would have eluted. The $K_d$ can be calculated from this information. This technique has been applied in Arthur Kornberg's laboratory using $[^3H] \beta$ in the column buffer to define the $K_d$ of a weak protein–protein interaction between the $\beta$ subunit and the Pol III* assembly (Pol III lacking $\beta$). \(^{21}\) Figure 2D shows an analysis of a weak interaction between the $\chi$ subunit of Pol III and an SSB–DNA complex using $[^3H]\chi$ in the column buffer. \(^{22}\)

**Exchange Rate Among Subunits of a Dimeric Protein**

How rapidly do dimers of identical subunits fall apart and come back together? For example, the protomers of the $\beta$ dimer form a closed ring, which must open to assemble around DNA. Do the monomer units of a $\beta$ dimer rapidly come apart and then reassociate, thereby trapping DNA inside? The rate of exchange of monomer units of a dimer cannot be measured by gel filtration as in Fig. 2C since it is always in the dimeric state. An assay to measure stability of the $\beta$ dimer, shown in Fig. 2E, uses a $[^3H]\beta$ dimer and a $\beta$ dimer that is genetically tagged with the hemagglutinin epitope (nine amino acids) at its C-terminus. \(^{23}\) Upon mixing the two $\beta$ dimers, samples of the reaction were immunoprecipitated at time intervals. As the “heterodimer” of one $[^3H]\beta$ and one epitope tagged-$\beta$ was formed, the amount of $[^3H]\beta$ in the coimmunoprecipitate increased. The results indicate that monomeric units of the $\beta$ dimer require a long time to dissociate even at 37°C. This assay can be exploited to study the influence of other proteins and of DNA and ATP on the stability of the $\beta$ dimer.

**Kinase Protection Assay**

We originally end-labeled proteins with $[^32P]$ to develop a “protein footprinting” assay for mapping the interactive surface of one protein with another (or with DNA), but this work is still in progress. However, we have developed a “kinase protection” assay. An example of this assay is shown in Fig. 2F in which $\beta^{PK}$ is incubated with or without the $\delta\delta'$ complex, a subassembly of the $\gamma$ complex that binds $\beta$. \(^{24}\) Then kinase is added and


the time course of phosphorylation is monitored by SDS–PAGE followed by autoradiography. The result shows that phosphorylation of βPK is almost completely blocked by the δδ' complex. The two C termini of the β dimer, where the kinase motif is located, extrude from the same face of the β dimer (see scheme in Fig. 2F). Hence, the ability of δδ' complex to block these sites from phosphorylation suggests that δδ' may interact with the "C-terminal" face of the β dimer.

Acknowledgments

We are grateful to Dr. Susan S. Taylor for the catalytic subunit of cAMP-dependent protein kinase. Supported by Public Health Service grant GM38839.

[33] Cycling of *Escherichia coli* DNA Polymerase III from One Sliding Clamp to Another: Model for Lagging Strand

*By Jennifer Turner and Mike O'Donnell*

Introduction

The multiprotein replicase of the *Escherichia coli* chromosome, DNA polymerase III holoenzyme (Pol III), achieves a tight ATP-activated grip on DNA through its ring-shaped clamp protein, the β dimer (β2). The β2 ring encircles DNA, thereby acting as a sliding clamp and continuously holds Pol III to the template for remarkably high speed and processivity. The model of a circular protein clamp riding along in back of the polymerase fits nicely with the continuous synthesis of the leading strand, but conceptually it would hinder the discontinuous mode on the lagging strand where Pol III must rapidly dissociate from the end of one Okazaki fragment to start another fragment at the next RNA primer (i.e., Fig. 1C).

Studies of Pol III showed that it was indeed very slow to replicate more than a stoichiometric number of primed templates. After Pol III completely replicated a circular single-stranded DNA (ssDNA) template, several