Eukaryotic DNA Polymerases

Sue Cotterill, St George's Hospital Medical School, London, UK Stephen Kearsey, Department of Zoology, University of Oxford, Oxford, UK

Advanced article

Article Contents

- Introduction
 - Structural Diversity of Eukaryotic Cellular Polymerases
- Polymerases Involved in Chromosome Replication
- Factors Involved in Polymerase Function at the Replication Fork
- Polymerases with Specialized Functions in Genome Replication
- Polymerases Involved in DNA Repair
- Polymerases Involved in Base Excision Repair
- Translesion Polymerases
- Polymerases Involved in NHEJ
- Polymerase with Unknown Functions
- Catalytic Function of Polymerases
- Assays
- Current Research Topics and Unanswered Questions

Online posting date: 15th December 2009

Deoxyribonucleic acid (DNA) is replicated and repaired by a family of enzymes called DNA polymerases. Eukaryotic cells have a diversity of these enzymes that, while sharing a common biochemical activity, are specialized for particular roles. Three polymerases are required for the replication of the nuclear genome, with pol α involved in priming and initial synthesis and pols δ and ε involved in bulk DNA replication. These polymerases are dependent on a large number of other proteins which unwind the DNA and perform other functions essential for efficient DNA synthesis. Polymerases are also involved in DNA repair and many repair-specific enzymes have been identified. Some repair polymerases can refill a gap generated by removal of damaged DNA, or copy a damaged template, allowing DNA synthesis to proceed across a damaged template. Repair polymerases can also have tissue-specific functions in lymphoid cells, where they contribute to somatic hypermutation of immunoglobulin genes.

ELS subject area: Biochemistry

How to cite:

Cotterill, Sue; and Kearsey, Stephen (December 2009) Eukaryotic DNA Polymerases. In: Encyclopedia of Life Sciences (ELS). John Wiley & Sons, Ltd: Chichester. DOI: 10.1002/9780470015902.a0001045.pub2

Introduction

The major function of deoxyribonucleic acid (DNA) polymerases is to replicate the genome and thus to allow transmission of genetic information from one generation to the next. In eukaryotic cells, this takes place during a discrete period (S phase) of interphase, and replicated chromosomes are subsequently segregated to daughter cells during mitosis. As well as replicating DNA, polymerases help to maintain the integrity of the genome by participating in various modes of DNA repair. DNA polymerases are also required for the replication of eukaryotic viruses. Some viruses, such as SV40, use host polymerases while others, such as adenovirus, encode their own polymerase; discussion of viral polymerases is outside the scope of this review. See also: Cell Cycle

The basic catalytic reaction of DNA polymerases is to effect semiconservative replication of DNA, using a singlestranded DNA chain as a template and four deoxynucleotides (deoxythymidine triphosphate (dTTP), deoxycytidine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP), deoxyadenosine triphosphate (dATP)) as precursors for DNA synthesis (Figure 1). The enzyme assembles the precursor nucleotides on the template to form a complementary DNA strand, selecting the incoming nucleotide using the base pair rules A • T and G • C. To start synthesis on a singlestranded DNA molecule, DNA polymerases need a primer. This is a length of ribonucleic acid (RNA) or DNA that is annealed to the single-stranded template. The primer provides a 3'-OH that can be extended by the polymerase; this configuration of the primer is important because polymerases can only extend a new chain in the 5' to 3' direction. If the



Figure 1 (a) Basic mechanism catalysed by DNA polymerases. Polymerization of nucleotides on the single-stranded template requires a primer (RNA or DNA) which provides a 3'-OH group to which the incoming nucleotide is joined and the direction of synthesis is thus 5' to 3'. Only nucleotides that correctly base pair with the templates strand (according to $A \bullet T$, $G \bullet C$ rules) are incorporated. One molecule of pyrophosphate (PP_i) is produced per nucleotide incorporated. (b) Steps in the polymerization reaction. The order of the reaction is binding to the template–primer, followed by binding of the dNTP. dNTP is cleaved at the α/β bond to give dNMP that is added onto the chain, PP_i is released, and the polymerase translocates along to the next 3' terminus or dissociates.

DNA template to be replicated is double-stranded, polymerase action still needs a primer and, in addition, requires other enzymes to unwind the double helix. **See also**: DNA Helicases; DNA Polymerase Fidelity Mechanisms; Polymerase Processivity: Measurement and Mechanisms

Structural Diversity of Eukaryotic Cellular Polymerases

Although all DNA polymerases share the same basic catalytic mechanism, at least 16 distinct polymerases have been identified in human cells (Hubscher et al., 2002; Prakash et al., 2005; Table 1). The reason for this diversity seems to be that polymerases have become specialized by modifying the characteristics of the polymerization reaction or by acquiring additional biochemical functions to tailor individual enzymes to particular tasks. In contrast to the single polymerase responsible for bacterial chromosome replication, three polymerases (α , δ and ε) are needed for eukaryotic chromosome replication (Table 1), and even more polymerase diversity is needed for DNA repair (Table 1). Polymerases involved in chromosome replication may also participate in repair pathways, such as nucleotide excision repair. Other types of repair synthesis involve specialized polymerases, some of which are proficient at replicating through a lesion, and this translesion synthesis (TLS) may be mutagenic. See also: Bacterial DNA Polymerase I

Some eukaryotic DNA polymerases consist of a single polypeptide chain, whereas others, such as those involved in chromosome replication, are composed of several

Table 1 Eukaryotic DNA polymerases		
Polymerase	Family	Function
θ (theta)	А	Base-excision repair?
v (nu)	А	?
γ (gamma)	А	Mitochondrial DNA replication
α (alpha)	В	Chromosome replication (initiation, Okazaki frag- ment priming)
δ (delta)	В	Chromosome replication (elongation, lagging strand synthesis) Nucleotide excision repair, mismatch repair
ε (epsilon)	В	Chromosome replication (elongation, leading strand synthesis) Nucleotide excision repair, mismatch repair?
ζ (zeta)	В	Translesion synthesis
ß (beta)	Х	Base-excision repair
λ (lambda)	X	Base-excision repair, non- homologous end joining
μ (mu)	Х	Nonhomologous end
TdT	Х	Nonhomologous end
η (eta)	Y	Translesion synthesis, error-free replication of CPDs
ι (iota)	Y	Translesion synthesis
к (kappa)	Y	Translesion synthesis, nucleotide-excision repair
Rev1	Y	Translesion synthesis
Telomerase	RT	Telomere maintenance

DNA polymerase σ (Trf4/5), originally implicated in chromosome cohesion (Wang *et al.*, 2000), is in fact a poly(A) RNA polymerase and has no DNA polymerase activity (Haracska *et al.*, 2005). Trf4 and Trf5 proteins of *Saccharomyces cerevisiae* exhibit poly(A) RNA polymerase activity but no DNA polymerase activity. CPD=cyclobutane pyrimidine dimmer.

different subunits. However, in each case one of the subunits is easily identifiable as containing the polymerase catalytic site and this allows classification of polymerases on the basis of amino acid sequence similarities. Eukaryotic DNA polymerases fall into five families, designated A, which also includes the archetypal *Escherichia coli* DNA polymerase I; B, which includes eukaryotic polymerases involved in bulk chromosome replication and RT, X and Y, which include enzymes involved in DNA repair or specialized types of replication (**Table 1**). On the basis of sequence comparisons, polymerases in different families may have limited similarity, but polymerases in families A and B appear to have an identical mechanism for the polymerization reaction (see the following section).

Polymerases Involved in Chromosome Replication

Chromosomes are replicated during the S phase of the cell cycle by three multisubunit polymerases – DNA pols α , δ and ε – which are found in all eukaryotes. The process of DNA replication involves initiation, which involves pol α , when DNA synthesis is activated at multiple replication origins in the chromosomes, and elongation, which involves extension by pols δ and ε of the short DNA chains generated during initiation by pol α . A summary of the properties of these polymerases is provided in the following sections; for detailed information on these polymerases refer to the comprehensive reviews by Hubscher *et al.* (2002); Garg and Burgers (2005) and Johnson and O'Donnell (2005).

Pol α

Only pol α has an associated primase activity capable of synthesizing short (approximately 10 nucleotide) RNA primers, and this function is associated with two of the four polymerase subunits. DNA pol α is therefore the only enzyme known to be involved in primer synthesis during initiation at the origins of replication. Also, the 5' to 3' directionality of DNA polymerase means that one strand of the DNA has to be synthesized discontinuously and therefore pol α is also required during the elongation step for the priming of synthesis of Okazaki fragments on the lagging strand. Following primer synthesis, DNA synthesis can occur, catalysed by the largest subunit, but since this polymerase does not have proofreading ability, synthesis is error-prone. The remaining subunit (B subunit) may have a role in regulating polymerase activity. After DNA pol α has synthesized a short (30–40 nucleotide) stretch of DNA, a process called polymerase switching takes place in which pol α is displaced from the template and synthesis by pols δ or ε takes over. See also: DNA Replication: Mammalian; Eukaryotic Chromosomes; Eukaryotic Replication Origins and Initiation of DNA Replication

Pol δ

Pol δ is a three- or four-subunit polymerase and probably functions at the lagging strands of the replication fork (Kunkel and Burgers, 2008). Unlike pol α , pol δ has proofreading activity, which is important for the highfidelity replication of chromosomes. Efficient synthesis of DNA by pol δ requires proliferating cell nuclear antigen (PCNA); pol δ interacts with PCNA via multiple interactions. Since PCNA forms a ring around the template, this enhances the processivity of the polymerase. See also: DNA Polymerase Fidelity Mechanisms; Eukaryotic Replication Fork.

As well as being involved in chromosome duplication, pol δ (and possibly pol ϵ) is involved in nucleotide excision and mismatch repair. This type of repair involves recognition of a damaged or mismatched nucleotide, followed by single-stranded incisions on each side of the damaged/ mismatched region. This allows removal of a short oligonucleotide (24–32 nucleotides) and the resulting singlestranded region can then be copied by pol δ . Ligation of the nick completes the repair.

Pol _ε

Pol ε consists of four subunits and probably functions at the leading strand (Kunkel and Burgers, 2008). Like pol δ , pol ϵ has a 3'-5' exonuclease activity that provides a proofreading function. Pol ε is a processive enzyme by itself and it is not clear whether it needs to interact with PCNA for function in vivo (reviewed in Garg and Burgers, 2005). Although pol ɛ is considered to be involved in bulk chromosome replication, the N-terminal region of the catalytic subunit, which contains the DNA polymerase and exonuclease motifs, is not essential for viability in yeasts, implying that pol δ can assume the synthetic role of pol ɛ (Kesti et al., 1999). DNA pol ɛ catalytic domains are dispensable for DNA replication, DNA repair and cell viability (Kesti et al., 1999; Feng and D'Urso, 2001). Schizosaccharomyces pombe cells lacking the *N*-terminal catalytic domains of DNA pol ε are viable but require the DNA damage checkpoint control (Feng and D'Urso, 2001). As well as physically replicating the DNA in the chromosomes, pol ε has been proposed to have a checkpoint role in budding yeast. This property resides in the noncatalytic C-terminal region of the large catalytic subunit, and may reflect the role the enzyme plays in the assembly of other replication factors at the replication fork. See also: Checkpoints in the Cell Cycle

Factors Involved in Polymerase Function at the Replication Fork

As can be seen from the preceding discussion, proper function of the replicative polymerases is dependent on a number of other proteins. The DNA in a cell does not exist as a naked single-stranded structure on which polymerases can freely function, but rather as a double-stranded helix in a protein-covered chromosome. Polymerase duplication of chromosomal DNA can therefore take place only after a number of factors have acted to allow the polymerase access to the DNA and create an environment conducive to polymerase activity. During initiation of replication, a whole network of proteins is involved in generating singlestranded DNA and loading polymerases onto the origin, although there is considerable uncertainty about how the replicative polymerases associate with chromatin (reviewed in Sclafani and Holzen, 2007). An initial step required for DNA replication is pre-replicative complex formation, when origin recognition complex (ORC), Cdc10-dependent transcript 1 (Cdt1) and cell division cycle 6 (Cdc6) assemble the minichromosomal maintenance 2-7 (Mcm2-7) helicase at the origins. Subsequently, activation of cyclin-dependent kinase (CDK) and Cdc7 kinase leads to association of other proteins with origins, such as Go-Ichi-Ni-San complex (GINS), Cdc45 and pols α and ϵ (reviewed in Scafani and Holzen, 2007). The early recruitment of pol ε reflects its probable role as the leading strand polymerase, so that it is in a position to take over from priming synthesis by pol α . Although both pols α and ε associate with origins at around the same time, it is possible that distinct pathways effect this recruitment (Pai et al., 2009). On the one hand, pol ε chromatin association may require interaction with Dpb11, which in turn interacts with other initiation factors, synthetically lethal with dpb11 (Sld2) and Sld3, in response to CDK activation (Zegerman and Diffley, 2007). On the other hand, pol α recruitment may require Mcm10 and acidic nucleoplasmic DNA-binding protein 1 (And1) (Ricke and Bielinsky, 2004; Zhu et al., 2007). Initiation by pol α requires DNA unwinding to take place, and the MCM protein complex most likely provides this helicase activity. It may be critical to control the chromatin association of pol α , since it has intrinsic primase activity and can potentially initiate replication *de novo*.

Following priming of synthesis, additional factors are required to allow polymerase switching, in which pols δ and ϵ take over from pol α and elongate the nascent DNA chain. This process involves the combined action of the replication protein A (RPA), replication factor C (RFC) and PCNA proteins (Waga and Stillman, 1998) and is best understood for switching to pol δ which is thought to occur on the lagging strand. When pol α encounters RPA on single-stranded DNA, the DNA pol α activity is inhibited. This allows binding of RFC, which recognizes the 3' end of the DNA formed by pol α . RFC has been termed 'clamp loader' as it functions to assemble PCNA, which forms a trimeric clamp, around the DNA template. PCNA links pols δ , and possibly ε , to the template and acts to increase the processivity of the polymerases by preventing premature dissociation from the template. On the leading strand, polymerase switching is only required once, but on the lagging strand it is needed every time DNA pol α initiates (100-200 nucleotides). On the lagging strand, synthesis is terminated when pol δ collides with the primer of the adjacent Okazaki fragment. Accessory factors such as the flap endonuclease (FEN) exonuclease, Dna2 exonuclease and DNA ligase are needed to remove the RNA primer and to seal the nick between the 3' end of the newly synthesized strand and the 5' end of the Okazaki fragment, allowing completion of DNA synthesis. A model showing the sequence of events involved in polymerase loading and DNA synthesis is available at www.dnareplication.net.

Polymerases with Specialized Functions in Genome Replication

Telomerase

Telomerase is another polymerase required to complete chromosome synthesis for maintaining telomeres at the ends of chromosomes (reviewed in Autexier and Lue, 2006). Telomeres are eroded during DNA replication since the lagging strand cannot be replicated from the very end of the chromosome. This erosion is counted by telomerase, which adds repeats of a short sequence to the ends of chromosomes. This enzyme is actually an RNA-directed DNA polymerase, and is unusual in that it is not template-directed but uses an internal RNA molecule to direct DNA synthesis. See also: Telomeres

Pol y

DNA pol γ is found in mitochondria and is required for replication and repair of the mitochondrial DNA. Pol γ consists of one catalytic subunit and two accessory subunits, all encoded in the nuclear genome. Mutations in pol γ are associated with a number of neurodegenerative diseases (reviewed in Chan and Copeland, 2009).

Polymerases Involved in DNA Repair

Most types of DNA repair involve the generation of a single-stranded region of DNA and therefore require the action of DNA polymerases to regenerate the doublestranded structure (Wood and Shivji, 1997). A diverse set of proteins is needed for recognition of the various types of damaged DNA and their subsequent processing, to provide a suitable substrate for DNA polymerases. The discussion here is restricted to polymerase action in the final synthetic stages of repair.

Enzymes from four different classes of polymerases (A, B, X and Y) have been shown to participate in DNA repair (Table 1). These polymerases use a variety of mechanisms to affect repair. A number of polymerases have been recently identified and characterized which are involved in the process of TLS (reviewed in Prakash et al., 2005). In this case, the polymerase can function at the replication fork, temporarily replacing a normal replicative polymerase which is incapable of copying the damaged base and allowing synthesis across the site of damage. Several such polymerases (see the following section) have been shown to be directed to damaged DNA by interaction with ubiquitylated PCNA. It is important to regulate the activity of translesion polymerases carefully, to avoid potentially mutagenic replication. This is because these polymerases copy undamaged DNA with poor fidelity, partly because they lack proofreading activity, and also because they can extend primers that are mispaired with the template, or can more readily incorporate nucleotides that do not form correct Watson-Crick base pairs with the template.

There has been considerable evolutionary expansion of TLS polymerases in vertebrates compared to simpler eukaryotes, possibly reflecting their roles in processes such as immunoglobulin gene hypermutation (reviewed in Sale *et al.*, 2009) and other tissue-specific functions. It may also reflect the fact that they are specialized to cope with replication across different types of lesions. Several TLS

polymerases have been seen to cooperate to allow the bypass of a DNA lesion, with the first polymerase inserting a base opposite the site of the lesion and a different enzyme extending the synthesis.

Other repair polymerases are specialized to incorporate a single nucleotide into the site of a lesion that has been removed (e.g. pol β in base excision repair (BER)) or perform template-independent synthesis (e.g. pols λ and μ in nonhomologous end joining). See also: DNA Damage; DNA Mismatch Repair: Eukaryotic; DNA Repair; DNA Repair by Reversal of Damage; Human Mismatch Repair: Defects and Predisposition to Cancer; Nucleotide Excision Repair in Eukaryotes

Polymerases Involved in Base Excision Repair

Pol β

In BER, removal of an altered base is followed by excision of a single abasic nucleotide. DNA pol β , a family X polymerase, then serves to fill in the missing nucleotide; this involves an activity in the *N*-terminal domain of the protein that removes the 5' phosphate remaining after nucleotide excision, followed by a polymerization reaction to fill in the missing nucleotide. This limited synthesis makes sense in terms of the biochemical properties of DNA pol β , which is distributive and has no proofreading activity. In a different mode of BER (termed 'long-patch'), a longer (2–10 nucleotide) stretch of DNA is removed, but it is unclear whether this is filled in by pol β , or by the replicative δ and ε polymerases. See also: Base Excision Repair, AP Endonucleases and DNA Glycosylases

Pol θ

Pol θ has a lower fidelity than other family A polymerases and is moderately processive (Arana *et al.*, 2008). It is capable of inserting a nucleotide opposite an abasic site and continuing synthesis. Pol θ may therefore participate in BER, overlapping in this function with pol β . Pol θ is also one of several error-prone polymerases that have been proposed to play a role in somatic hypermutation (Masuda *et al.*, 2005; reviewed in Sale *et al.*, 2009). This process allows mutation of antibody genes at a rate that is 10⁶ times higher than background levels and is important for generating antibody diversity. **See also**: Immunoglobulin Gene Rearrangements; Somatic Hypermutation in Antibody Evolution

Translesion Polymerases

Pol η

The importance of the family Y polymerase, pol η , is demonstrated by the genetic disease xeroderma

pigmentosum variant (XPV) in which affected individuals lack the enzyme and as a consequence suffer a high rate of sunlight-induced skin cancer (reviewed in Prakash et al., 2005). This defect seems to arise from the requirement for pol η in copying T–T photodimers, which are a product of UV irradiation of DNA. Normal replicative polymerases are impeded by these photodimers, but pol n correctly incorporates two As opposite each mutant T-T site, thus allowing error-free replication. Pol n also allows error-free bypass of 8-oxoguanine (Haracska et al., 2000), but it has a high error rate when copying undamaged nucleotides (10^{-2}) to 10^{-3} compared to 10^{-6} for pol δ). The polymerase appears to extend mismatched primer termini inefficiently which could allow a proof reading polymerase such as δ to remove the mismatched base, thus mitigating its high error rate. Recent evidence suggests that pol η interacts with PCNA, allowing pol η to be targeted to the replication fork, and that ubiquitylation of PCNA may be important for allowing pol η to take over from the replicative DNA polymerase at the site of a DNA lesion (Acharya et al., 2008). The error-prone nature of pol n DNA synthesis may also be exploited to generate mutations in antibody genes.

Pol ζ

Pol ζ is a family B polymerase which appears to contribute to TLS by its ability to extend a primer–template in the vicinity of a DNA lesion (reviewed in Gan *et al.*, 2008). Since some bypass polymerases, such as pol η , can efficiently incorporate opposite a lesion but may not be able to extend from the incorporated base, pol ζ extends synthesis and thus aids complete replication over the lesion. Pol ζ allows error-free replication past thymine glycol (Johnson *et al.*, 2003), and this may help to explain embryonic lethality seen in pol ζ deficient mice.

Polι

Pol ι is family Y polymerase which has been shown to be able to bypass some types of DNA damage *in vitro* (reviewed in Vidal and Woodgate, 2009). It is an unusual polymerase as its error rate is dependent on the template nucleotide. For instance, it is moderately accurate when the template nucleotide is A, but misincorporates G rather than A when the template is T. It is possible that this is related to its use of Hoogsteen base pairing for DNA synthesis. The enzyme lacks processivity and is not able to continue synthesis from the first nucleotide inserted. Its biological function is still unclear, and mice lacking pol ι do not show any dramatic phenotypes.

Pol ĸ

The family Y polymerase pol κ , like pol ζ , has been implicated in extending from the nucleotide incorporated opposite a damaged site by another polymerase. It is not an essential enzyme in mice. Pol κ has also been implicated in nucleotide excision repair, and in TLS past interstrand crosslinks (Minko *et al.*, 2008).

Rev1

Bypass of an abasic site can be effected by the family Y polymerase Rev1, which incorporates a C in a templatedependent (but not template-directed) manner. Rev1 can also incorporate C against all four nucleotides (Haracska *et al.*, 2002). Rev1 contains an *N*-terminal Brca1 C-terminus (BRCT) domain that is important for allowing interaction with PCNA (Guo *et al.*, 2006). Genetic evidence suggests that this protein may contribute to TLS in a mechanism that does not require its catalytic function, presumably as it may affect the recruitment of other proteins at the damage site (Haracska *et al.*, 2001). Consistent with this, a *C*-terminal region of Rev1 is able to interact with a number of other family Y polymerases. Rev1 appears to contribute to immunoglobulin hypermutation (Sale *et al.*, 2009).

Polymerases Involved in NHEJ

Pol λ , Pol μ and TdT

These family X polymerases function in nonhomologous end joining, allowing repair of double-strand breaks (Ma *et al.*, 2004). They have an unusual property of being able to perform template-independent extensions of 3' ends and this is likely to be important to provide microhomology between broken ends, facilitating their ligation and joining. These polymerases have also been implicated in V(D)J recombination in the immune system. Pol λ may also function in BER (Braithwaite *et al.*, 2005).

Polymerase with Unknown Functions

Pol v

Pol v lacks proofreading activity and shows lower fidelity than other family A polymerases. The most common error generated by pol v involves the insertion of a T opposite a template G, and in some sequence contexts the error rate can even exceed 10% (Arana *et al.*, 2007). Although the precise biological function of this enzyme is unknown, it is orthologous to the *Drosophila* mus308 protein which has a role in the repair of interstrand crosslinks (Marini *et al.*, 2003).

Catalytic Function of Polymerases

Structural analyses of family A and family B polymerases have revealed similarity in their structures and catalytic mechanisms (Steitz, 1999; Patel and Loeb, 2001). The enzyme can be compared to a cupped right hand, with the palm providing the catalytic amino acids, the fingers binding the incoming nucleotide and the single-stranded template, and the thumb binding the double-stranded DNA (Figure 2). The palm domain appears to be





Figure 2 Structure of RB69 polymerase. RB69 is a prokaryotic family B polymerase, and is therefore likely to have a structure representative of eukaryotic replicative polymerases (α , δ , ε ; Franklin *et al.*, 2001). (a) Polymerization mode. DNA template–primer (pink-orange) first binds to the polymerase thumb domain, followed by binding of a nucleotide triphosphate (blue), complementary to the template base immediately adjacent to the 3'-OH of the primer. On binding the dNTP, the finger domain (shown here in the open conformation) rotates (black arrow) towards the palm domain to form a closed ternary complex. This facilitates phosphade of the incoming dNTP. (b) In the exonuclease (proof-reading) mode, the thumb tip rotates to partition the DNA to the exonuclease site (shown in yellow), allowing removal of a noncomplementary base.

homologous for the A and B families, and while the finger and thumb domains are not, they show analogous features. Both family A and family B polymerases show a conformational change on DNA binding that involves rotation of the DNA-binding thumb domain towards the catalytic palm domain. On binding of the deoxyriboneucleotide triphosphate (dNTP), the polymerase then adopts a 'closed conformation' in which the nucleotide-binding site closes up, and the incoming dNTP becomes enclosed in a pocket with hydrophobic residues around the base and ribose and a hydrophilic environment around the triphosphate. Recent work has mapped in more detail the contact points of the nucleotide to two aspartic acid residues in the active site that are widely conserved in both DNA and RNA polymerases. These make contact with two magnesium ions that enter the binding cleft associated with phosphates of the incoming nucleotide. These metal ions are critical for catalysis of the polymerization reaction, and function by promoting the nucleophilic attack of the primer 3'-OH to the α -phosphate of the incoming dNTP. This closed pocket conformation is only possible if the incoming nucleotide achieves correct Watson-Crick pairing with the template, thus helping to promote fidelity of the enzyme.

As mentioned in the preceding section, a number of the replicative polymerases have, in addition to the polymerase activity, an exonuclease activity that is involved in proofreading – the removal of an inappropriate nucleotide that does not have correct Watson-Crick pairing with the template. Analysis of the crystal structure has also been able to shed light on this aspect of polymerase function. The exonuclease domain is not within the polymerization active site but is about 30–40 Å away. If the wrong nucleotide is incorporated onto the growing chain, it slows polymerization, probably because the 3'-OH of the primer to which the next nucleotide must be added is misorientated. This allows time for the primer terminus to move into the exonuclease active site. The DNA loses contact with the polymerization cleft but remains attached to the polymerase via the thumb domain, which rotates allowing movement of the DNA chain into the exonuclease domain. The fact that the DNA template does not lose contact with the polymerase at any time during this operation allows misincorporations to be removed efficiently and this mechanism therefore contributes to both the fidelity and processivity of the polymerase reaction. See also: DNA Polymerase Fidelity Mechanisms

Assays

The incorporation of nucleotides easily lends itself to an assay for the enzyme. All assays for DNA polymerases involve measurement of the incorporation of labelled nucleotides into a primed DNA template. Various templates have been used. The earliest was activated calf thymus DNA, where single-stranded gaps are made in a double-stranded template by DNase I. In this case, the ability of the enzyme to fill in the gaps (on average 30–40 bp) is measured. More modern assays use single-stranded DNA molecules (such as bacteriophage M13) with a single primer, or use homopolymer type substrates such as poly(dA) sparsely primed with dT. These templates have an additional advantage in that the ability of a polymerase to synthesize a long stretch of DNA, or to pass a particular sequence or DNA structure, can also be ascertained.

Biochemical characterization of polymerase activity *in vitro* routinely measures three parameters: the rate of polymerization, fidelity and processivity. The fidelity for polymerases with a proofreading activity is generally around 1 error per 10^{5} – 10^{6} nucleotides incorporated. Processivity can be very variable depending on the polymerase and is often increased by accessory factors.

Current Research Topics and Unanswered Questions

The last few years have seen some progress on understanding the interactions and recruitment of polymerases at the replication fork. Repair polymerases are also beginning to be better understood. The *in vitro* synthesis properties of many of them are now quite well defined and in some cases clues as to their cellular function have been determined. However, many questions still remain to be answered about both replicative and repair polymerases. For example, the exact mechanisms by which replicative polymerases are recruited to chromatin require clarification, and it also needs to be determined whether the assignment of pol ε to the leading strand and pol δ to the lagging strand holds up for all areas of the genome and in all organisms. Structural studies on the repair polymerase families need to be extended. In addition, more information needs to be gathered about their precise cellular functions and how the correct polymerase is recruited to a specific lesion. Given the ongoing interest in all of these areas, it is likely that the answers to some of these questions will soon be available.

References

- Acharya N, Yoon JH, Gali H *et al.* (2008) Roles of PCNA-binding and ubiquitin-binding domains in human DNA polymerase {eta} in translesion DNA synthesis. *Proceedings of the National Academy of Sciences of the USA* **105**: 17724–17729.
- Arana ME, Seki M, Wood RD, Rogozin IB and Kunkel TA (2008) Low-fidelity DNA synthesis by human DNA polymerase theta. *Nucleic Acids Research* 36: 3847–3856.
- Arana ME, Takata K, Garcia-Diaz M, Wood RD and Kunkel TA (2007) A unique error signature for human DNA polymerase nu. DNA Repair (Amsterdam) 6: 213–223.
- Autexier C and Lue NF (2006) The structure and function of telomerase reverse transcriptase. *Annual Review of Biochemistry* 75: 493–517.
- Braithwaite EK, Prasad R, Shock DD et al. (2005) DNA polymerase lambda mediates a back-up base excision repair activity in extracts of mouse embryonic fibroblasts. *Journal of Biological Chemistry* 280: 18469–18475.
- Chan SS and Copeland WC (2009) DNA polymerase gamma and mitochondrial disease: understanding the consequence of POLG mutations. *Biochimica et Biophysica Acta*. **1787**(5): 312– 319
- Feng W and D'Urso G (2001) *Schizosaccharomyces pombe* cells lacking the amino-terminal catalytic domains of DNA

polymerase epsilon are viable but require the DNA damage checkpoint control. *Molecular and Cellular Biology* **21**: 4495–4504.

- Franklin MC, Wang J and Steitz TA (2001) Structure of the replicating complex of a pol alpha family DNA polymerase. *Cell* **105**: 657–667.
- Gan GN, Wittschieben JP, Wittschieben BO and Wood RD (2008) DNA polymerase zeta (pol zeta) in higher eukaryotes. *Cell Research* **18**: 174–183.
- Garg P and Burgers PM (2005) DNA polymerases that propagate the eukaryotic DNA replication fork. *Critical Reviews in Biochemistry and Molecular Biology* **40**: 115–128.
- Guo C, Sonoda E, Tang TS *et al.* (2006) REV1 protein interacts with PCNA: significance of the REV1 BRCT domain *in vitro* and *in vivo*. *Molecular Cell* **23**: 265–271.
- Haracska L, Johnson RE, Prakash L and Prakash S (2005) Trf4 and Trf5 proteins of *Saccharomyces cerevisiae* exhibit poly(A) RNA polymerase activity but no DNA polymerase activity. *Molecular and Cellular Biology* 25: 10183–10189.
- Haracska L, Prakash S and Prakash L (2002) Yeast Rev1 protein is a G template-specific DNA polymerase. *Journal of Biological Chemistry* 277: 15546–15551.
- Haracska L, Unk I, Johnson RE *et al.* (2001) Roles of yeast DNA polymerases delta and zeta and of Rev1 in the bypass of abasic sites. *Genes & Development* 15: 945–954.
- Haracska L, Yu SL, Johnson RE, Prakash L and Prakash S (2000) Efficient and accurate replication in the presence of 7,8-dihydro-8-oxoguanine by DNA polymerase eta. *Nature Genetics* 25: 458–461.
- Hubscher U, Maga G and Spadari S (2002) Eukaryotic DNA polymerases. *Annual Review of Biochemistry* **71**: 133–163.
- Johnson A and O'Donnell M (2005) Cellular DNA replicases: components and dynamics at the replication fork. *Annual Review of Biochemistry* **74**: 283–315.
- Johnson RE, Yu SL, Prakash S and Prakash L (2003) Yeast DNA polymerase zeta (zeta) is essential for error-free replication past thymine glycol. *Genes & Development* 17: 77–87.
- Kesti T, Flick K, Keranen S, Syvaoja JE and Wittenberg C (1999) DNA polymerase epsilon catalytic domains are dispensable for DNA replication, DNA repair, and cell viability. *Molecular Cell* 3: 679–685.
- Kunkel TA and Burgers PM (2008) Dividing the workload at a eukaryotic replication fork. *Trends in Cell Biology* **18**: 521–527.
- Ma Y, Lu H, Tippin B *et al.* (2004) A biochemically defined system for mammalian nonhomologous DNA end joining. *Molecular Cell* **16**: 701–713.
- Marini F, Kim N, Schuffert A and Wood RD (2003) POLN, a nuclear PolA family DNA polymerase homologous to the DNA cross-link sensitivity protein Mus308. *Journal of Biological Chemistry* **278**: 32014–32019.
- Masuda K, Ouchida R, Takeuchi A *et al.* (2005) DNA polymerase theta contributes to the generation of C/G mutations during somatic hypermutation of Ig genes. *Proceedings of the National Academy of Sciences of the USA* **102**: 13986–13991.
- Minko IG, Harbut MB, Kozekov ID et al. (2008) Role for DNA polymerase kappa in the processing of N2-N2-guanine interstrand cross-links. *Journal of Biological Chemistry* 283: 17075– 17082.

- Pai CC, García I, Wang SW *et al.* (2009) GINS inactivation phenotypes reveal two pathways for chromatin association of replicative α and ε DNA polymerases in fission yeast. *Molecular Biology of the Cell* **20**: 1213–1222.
- Patel PH and Loeb LA (2001) Getting a grip on how DNA polymerases function. *Nature Structural Biology* **8**: 656–659.
- Prakash S, Johnson RE and Prakash L (2005) Eukaryotic translesion synthesis DNA polymerases: specificity of structure and function. *Annual Review of Biochemistry* 74: 317–353.
- Ricke RM and Bielinsky AK (2004) Mcm10 regulates the stability and chromatin association of DNA polymerase-alpha. *Molecular Cell* **16**: 173–185.
- Sale JE, Batters C, Edmunds CE et al. (2009) Timing matters: errorprone gap filling and translesion synthesis in immunoglobulin gene hypermutation. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **364**: 595–603.
- Sclafani RA and Holzen TM (2007) Cell cycle regulation of DNA replication. Annual Review of Genetics 41: 237–280.
- Steitz TA (1999) DNA polymerases: structural diversity and common mechanisms. *Journal of Biological Chemistry* 274: 17395–17398.
- Vidal AE and Woodgate R (2009) Insights into the cellular role of enigmatic DNA polymerase iota. *DNA Repair (Amsterdam)* 8: 420–423.
- Waga S and Stillman B (1998) The DNA replication fork in eukaryotic cells. *Annual Review of Biochemistry* 67: 721–751.
- Wang Z, Castano IB, De Las Penas A, Adams C and Christman MF (2000) Pol kappa: a DNA polymerase required for sister chromatid cohesion. *Science* 289: 774–779.
- Wood RD and Shivji MK (1997) Which DNA polymerases are used for DNA-repair in eukaryotes? *Carcinogenesis* 18: 605– 610.
- Zegerman P and Diffley JF (2007) Phosphorylation of Sld2 and Sld3 by cyclin-dependent kinases promotes DNA replication in budding yeast. *Nature* **445**: 281–285.
- Zhu W, Ukomadu C, Jha S *et al.* (2007) Mcm10 and And-1/CTF4 recruit DNA polymerase alpha to chromatin for initiation of DNA replication. *Genes & Development* **21**: 2288–2299.

Further Reading

- Burgers PM (2009) Polymerase dynamics at the eukaryotic DNA replication fork. *Journal of Biological Chemistry* **284**: 4041–4045.
- Cotterill S (ed.) (1998) DNA Replication, A Practical Approach. Oxford, UK: Oxford University Press.
- Cotterill S and Kearsey S (2008) DNA Replication: a database of information and resources for the eukaryotic DNA replication community. *Nucleic Acids Research* **37**(Database issue): D837–D839.
- DePamphilis ML (ed.) (2006) DNA Replication and Human Disease. New York, NY: Cold Spring Harbor Press.
- Stillman B (1994) Smart machines at the DNA replication fork. *Cell* **78**: 725–728.
- Vengrova S and Dalgaard JZ (eds) (2009) DNA Replication, Methods and Protocols. New York, NY: Humana Press.