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Function of DNA Polymerase α in a Replication Fork and its Putative Roles in Genomic Stability and Eukaryotic Evolution

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1. Introduction

DNA replication takes place during S phase (Kornberg & Baker, 1992). In eukaryotic cells, DNA replication is performed through the concerned action of plural DNA polymerases with their accessory proteins (Wang, 1991; Hübscher et al., 2002; Burgers, 2009). Eukaryotic cells contain three replicative DNA polymerases, named DNA polymerases α, δ, and ε, mitochondrial DNA polymerase γ, and at least 12 non-replicative DNA polymerases, named DNA polymerases β, ζ, η, θ, ι, κ, λ, μ, and ν, terminal deoxynucleotidyl transferase (TdT), and Rev1 (Hübscher et al., 2002; Livneh et al., 2010). DNA replication progresses according to the catalytic activity of three replicative DNA polymerases (pol-) α, δ, and ε, cooperatively (Burgers, 2009). Pol-α is necessary for the onset of DNA synthesis, which synthesizes RNA-DNA primer in both leading and lagging strands (Wang, 1991; Hübscher et al., 2002; Burgers, 2009). Pol-δ is probably involved in the synthesis of Okazaki fragment in the lagging strand, and pol-ε is thought to synthesize long DNA strand in the leading strand (Pavlov et al., 2006a; Pavlov et al., 2006b; Pursell et al., 2007) (Fig. 1).

Pol-α is the first eukaryotic DNA polymerase ever discovered (Bollum, 1960). Its complex type with DNA primase (pol-α-primase complex) is the only DNA polymerase that can initiate DNA synthesis *de novo*. Therefore, pol-α is an important enzyme for cell survival (Murakami et al., 1985; Budd & Campbell, 1987; Takada-Takayama et al., 1991). Pol-α-primase complex comprises four subunits, each of which is highly conserved in eukaryotes from yeast to human (Wang, 1991). The function of this 'classical' DNA polymerase at DNA replication reaction has been well established. Primase is an RNA polymerase. It is able to synthesize RNA primer, following DNA synthesis by the catalytic subunit of pol-α. Almost all DNA replication researchers consider its role in DNA replication reaction as a mere 'initiator' of DNA chain elongation. Nevertheless, several recent examinations of this 'classical' DNA polymerase have revealed its heretofore unknown but important roles in DNA replication. They have revealed a link between DNA replication and other cellular dynamism, in addition to advancing the evolution of eukarya. According to this recent progress, I describe several topics related to eukaryotic pol-α in this chapter, not only as an 'initiator', but also as a 'key regulator'.

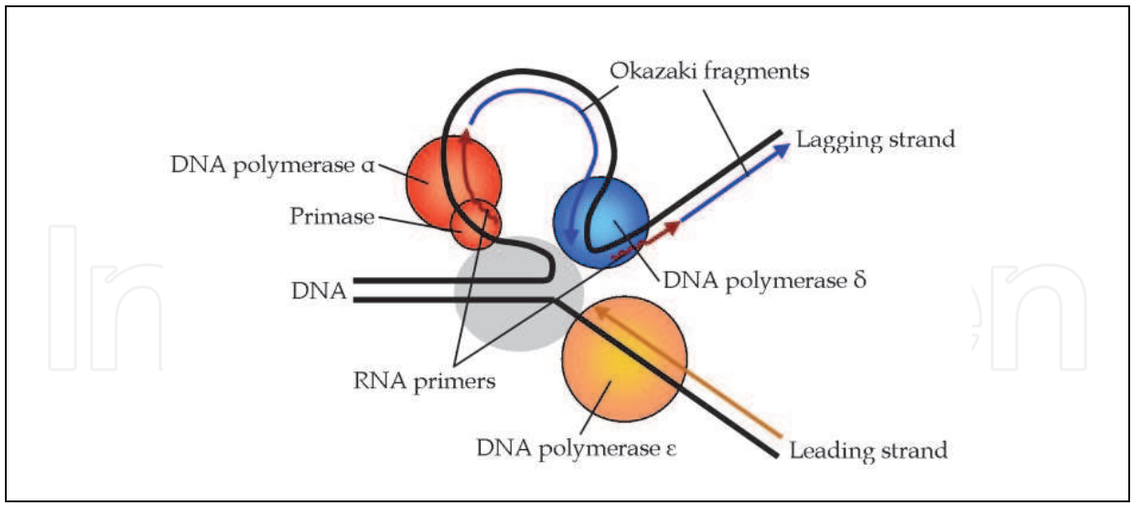

Fig. 1. Typical model of eukaryotic DNA replication fork.

2. Structure of pol-α-primase complex

In 1960, pol-α became the first discovered eukaryotic DNA polymerase from calf thymus (Bollum, 1960). Eukaryotic pol-α was found to be tightly associated with DNA primase (Yagura et al., 1982; Yagura et al., 1983; Frick & Richardson, 2001). Now pol-α has been purified as a multisubunit complex that is tightly associated with DNA primase activity from various eukaryotic cells and tissues, the so-called pol-α-primase complex (Wang, 1991; Frick & Richardson, 2001; Hübscher et al., 2002).

Pol-α-primase complex comprises four subunits: p180 (in mouse and human), the largest subunit associated with catalytic polymerase activity; p68 (in mouse and human) and p54 (in mouse, p58 in human), the second-largest and third-largest subunits whose function has been investigated to date; and p46 (in mouse, p48 in human), a primase subunit (Miyazawa et al., 1993). Subunit compositions of pol-α-primase complex in each typical organism are presented in Table 1.

In calf, pol-α-primase complex can be immunopurified from calf thymus using anti-pol-α monoclonal antibody SJK287-38 or MT-17 (Nasheuer & Grosse, 1987; Tamai et al., 1988; Takemura et al., 1997). Purified calf pol-α comprises at least 3 or 4 subunits (Nasheuer & Grosse, 1987; Tamai et al., 1988; Takemura et al., 1997). Reportedly, the immunopurified calf thymus pol-α-primase complex using monoclonal antibody SJK287-38 has 148-180, 73, 59, and 48 kDa subunits (Nasheuer & Grosse, 1987). However, Tamai et al. reported that the immunopurified calf pol-α-primase complex using MT-17 consists of at least three subunits, which have 140, 50, and 47 kDa, respectively (Tamai et al., 1988). It was also reported that the immunopurified pol-α-primase complex using MT-17 from calf thymus had 4 subunits with 140, 73, 50, and 47 kDa (Takemura et al., 1997), corresponding with the subunit composition in human and mouse (Mizuno et al., 1999; see also Mizuno's chapter).

The subunits of pol-α-primase complex in the yeast *Saccharomyces cerevisiae* are 167, 79, 62, and 48 kDa polypeptides (Hübscher et al., 2002; Biswas et al., 2003). The pol-α-primase complex from *Drosophila melanogaster* consists of 182, 73, 60, and 50 kDa subunits (Kaguni et al., 1983; Cotterill et al., 1987). The estimated roles of each subunit of pol-α is summarized in Fig. 2.

	Human	Mouse	Calf	Fly	Budding yeast	Fission yeast
Catalytic subunit	180	180	140	182	167	170
2nd-largest subunit	68	68	73	73	79	66
3rd-largest subunit	58	54	50	60	62	55
Primase subunit	48	46	47	50	48	45

Table 1. Subunit compositions (kDa) of pol-α-primase complex in six organisms.

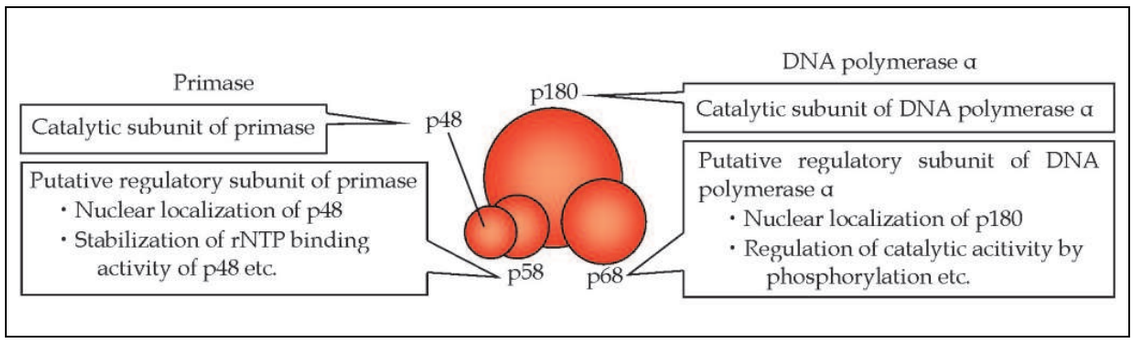

Fig. 2. The estimated roles of each subunit.

Catalytic subunit of pol-α is thought to bind several carbohydrate chains. Reportedly, several lectins such as Concanavalin A bound human catalytic subunit of pol-α (p180), and that ricin from castor beans bound and inhibited pol-α, suggesting that the p180 subunit has carbohydrate chains recognized by these lectins (Bhattacharya et al., 1979; Hsi et al., 1990). Because tunicamycin, which inhibits the glycosylation of protein, has been reported to decrease the activity of pol-α (Bhattacharya & Basu, 1982), it is suggested that the carbohydrate chain on pol-α is important for its activity. However, the detailed roles of carbohydrate chains on p180 subunit have not been elucidated to date.

In the p180 catalytic subunit, three separate domains were identified: an N-terminal domain (amino acids 1–329), a central domain (amino acids 330–1279), and a C-terminal domain (amino acids 1235–1465) (Hübscher et al., 2002). Immunohistochemical analysis using several mutant subunits whose abilities of interaction with other subunits were absent has revealed the mechanism of the subunit assembly, domain structure, and stepwise formation of pol-α-primase complex at the cellular level (Mizuno et al. 1999). The N-terminal domain is thought to be involved in the catalytic activity and the assembly of tetrameric complex, but the detailed roles have not been elucidated. The central domain contains conserved regions among B-family DNA polymerases, which are responsible for dNTP binding, template DNA binding, and the catalytic activity forming a phosphodiester bond (Hübscher et al., 2002). The C-terminal domain was thought to be necessary for its interaction with both p68 subunit and p54/p46 primase subunits (Mizuno et al., 1998; Mizuno et al., 1999) (see also Mizuno's chapter). Recently, the 3D architecture of *Saccharomyces cerevisiae* pol-α was constructed (Klinge et al., 2009). According to this architecture, the combination between X-ray crystallography and electron microscopy revealed the structural properties of the

subunit interaction, especially between p180 and p68 subunit. It was revealed that the C-terminal domain of the catalytic subunit of yeast pol-α contains two C4 zinc-binding motifs and interacts with the second-largest subunit through the α2 helix (Klinge et al., 2009; Johansson & MacNeil, 2010).

Although the tetrameric complex of pol-α-primase is apparently tightly formed, it has some flexibility. As described above, calf thymus pol-α-primase complex is sometimes purified in the trimeric complex, which lacks the second-largest subunit (Tamai et al., 1988). In the case of the tetrameric complex purified, the amounts of the second-largest subunit are sometimes lower than those of other subunits (Nasheuer & Grosse, 1987; Takemura et al., 1997). It is particularly interesting that Mizuno et al. found that there are many 'free' second-largest (p68) subunit in COS-1 cells, in addition to that in tetrameric pol-α-primase complex (Mizuno et al., 1999). In *Coprinus cinereus*, one basidiomycete, pol-α-primase complex has been reported to dissociate, and the catalytic subunit (p135 in *Coprinus cinereus*) has been reported to be purified detached from other subunits during meiosis (Sawado & Sakaguchi, 1997; Namekawa et al., 2003). These findings suggest that pol-α-primase complex tightly forms in the DNA replication initiation step at the replication fork, and dissociates in other phases or function, before or after DNA replication reaction.

3. Function of pol-α in DNA replication initiation

According to the current models, the pol-α-primase complex initiates RNA/DNA primer synthesis on both leading and lagging strands (Waga & Stillman, 1998; Burgers 2009). The primase subunit of the complex synthesizes RNA primer with subsequent short DNA primer synthesis by the catalytic subunit of the complex. In one putative model, pol-α is switched to another processive pol-ε on the leading strand, and to pol-δ for complete Okazaki fragment synthesis on the lagging strand.

Pol-δ and pol-ε catalytic subunits are known to both contain a 3′-5′ proofreading exonuclease domain. Therefore, they have 3′-5′ exonuclease activity, otherwise pol-α catalytic subunit has no intrinsic exonuclease activity (Shevelev & Hübscher, 2002), except for the study of *Drosophila melanogaster* and recombinant *Saccharomyces cerevisiae* pol-α (Cotterill et al., 1987; Brooke et al., 1991). Because of the lack of proofreading activity, the initial deoxyribonucleotides in each Okazaki fragment and the leading strand are expected to contain misincorporated bases, which are able to cause mutation. Instead of the lack of 3′-5′ exonuclease activity, previous reports describe nuclear factors that are thought to be responsible for proofreading, thereby covering a deficit of intrinsic exonuclease of pol-α, or which have been co-purified with pol-α-primase complex (Bialek & Grosse, 1993; Brown et al., 2002). It was shown that the p53, a tumour suppressor protein, contains an intrinsic 3′-5′ exonuclease activity, and physically binds to pol-α (Mummenbrauer et al., 1996; Huang, 1998; Melle & Nasheuer, 2002). It remains unclear whether these nuclear factors work as 3′-5′ exonuclease substituted to the lost exonuclease activity of pol-α or not. A recent model suggests that the errors that occurred on short DNA primer by pol-α are removed by other proofreading (or editing) mechanisms. A possible model is that the errors made by pol-α are edited out by FEN1, flap endonuclease 1, during a process that removes RNA primer (Bae et al., 2001). This model is supported by a finding that the yeast cells carrying FEN1 endonuclease-defective mutation had a 25-fold increase in rates of base substitution, and mice carrying the FEN1 mutation caused in cancer (Zheng et al., 2007; Zheng & Shen, 2011).

Another model is that the intrinsic 3′-5′ exonuclease activity of pol-δ proofreads errors made by pol-α (Pavlov et al., 2006b).

To date, various studies have examined the link mechanism between the cell cycle and the DNA replication progression. Phosphorylation of proteins by cdk-cyclin families as cell cycle regulators is the most important mechanism to regulate the cell cycle. The activities of cdk2/cyclin E and cdk2/cyclin A complexes respectively peak in G_1 and S/G_2 phases of the cell cycle (Dulić et al., 1992; Koff et al., 1992; Rosenblatt et al., 1992). The cell cycle-dependent phosphorylation on pol-α is also found by several research groups. Catalytic p180 subunit of pol-α is reported to be phosphorylated throughout the cell cycle and is hyper-phosphorylated in the G_2/M phase (Nasheuer et al., 1991). The hyper-phosphorylated type of pol-α has lower affinity for single-stranded DNA (Nasheuer et al., 1991), suggesting that the phosphorylation of pol-α suppresses the activity of pol-α in its DNA synthesis via change in its conformation from active to inactive. The second-largest subunit is also shown to be phosphorylated in a cell cycle-dependent manner (Nasheuer et al., 1991). These subunits are phosphorylated by both cdk2/cyclin E and cdk2/cyclin A (Voitenleitner et al., 1999). Reportedly, the pol-α activity was decreased as a result of the phosphorylation of the second-largest subunit (Voitenleitner et al., 1999). It is particularly interesting that cdk2/cyclin A executes both stimulatory and inhibitory effects on pol-α activity in initiating *in vitro* DNA replication, which is putatively caused by distinct phosphorylation events on pol-α by cdk2/cyclin A (Schub et al., 2001). In yeast, dephosphorylation of the second-largest subunit occurs while cells exit mitosis (Foiani et al., 1995). The second-largest subunit is thought to have a regulatory role in an early stage of S phase. It is reportedly associated with the origin recognition complex (ORC) for initiation of DNA replication (Uchiyama & Wang). Huang et al. also reported that the interaction of the second-largest subunit with both catalytic p180 subunit and helicase is necessary for the SV40 primosome activity (Huang et al., 2010). The author and colleagues reported that the interaction of the catalytic p180 subunit of pol-α with hyper-phosphorylated retinoblastoma protein is regulated by the phosphorylation of the second-largest subunit (Takemura et al., 2006). These results cumulatively suggest that the second-largest subunit of pol-α regulates various stages of the activity of pol-α, depending upon its phosphorylation status (Fig. 2).

4. Various proteins bound to pol-α

Although the mechanisms that regulate DNA polymerases and DNA replication are not yet fully understood, *in vitro* DNA replication systems using tumour viruses such as simian virus 40 (SV40) have provided a useful tool for study in this area (Kelly, 1988; Hurwitz et al., 1990). SV40 DNA replication is accomplished by DNA replication machinery of the host cultured cells with the assistance of a single viral protein, large T antigen, which unwinds the double DNA strand at replication origin, thereby forming a preinitiation complex (Dean et al., 1987; Dodson et al., 1987; Stillman, 1989; Tsurimoto et al., 1990; Collins & Kelly, 1991; Murakami et al., 1992). With these *in vitro* DNA replication studies as a start, many studies of accessory factors associated with pol-α have been conducted to date (Fig. 3).

SV40 large T antigen can form a complex with pol-α, and stimulate its activity (Smale & Tjian, 1986; Dornreiter et al., 1990; Collins & Kelly, 1991; Dornreiter et al., 1992). Although DNA polymerase α has been shown to exist in the cell nucleus throughout the cell cycle (Nakamura et al., 1984), it might act only in the S phase. Therefore, pol-α might be regulated post-translationally by some nuclear factors. A number of stimulatory / accessory proteins

have been reported in addition to SV40 large T antigen. C-factor (C_1C_2 factor), microtubule-associated protein (MAP)-2, and factor T were, respectively, found to interact with and activate pol-α (Lamothe et al., 1981; Pritchard & DePamphilis, 1983; Pritchard et al., 1983; Yoshida et al., 1989; Shioda et al., 1991). CDC45 were bound to pol-α, by which DNA polymerase α is loaded onto the chromatin (Mimura & Takisawa, 1998). Chk1, an intra-S and DNA damage checkpoint protein, is also reported to bind physically with pol-α, suggesting that pol-α is also an important component of the signal transduction cascade activating the checkpoint (Toricani et al., 2009).

Simbulan et al. have reported that the poly (ADP-ribose) polymerase (PARP) is able to stimulate the activity of pol-α, and that auto-poly(ADP-ribosyl)ation of PARP itself decreases its stimulatory activity (Simbulan et al., 1993). Actually, PARP is known to post-translationally modulate target proteins by transfer and polymerization of ADP-ribose, using NAD as substrates (Miwa & Masutani, 2007). PARP (especially PARP-1) was found in the nuclei and was shown to be activated by DNA strand breaks (Miwa & Masutani, 2007). Although the role of PARP in DNA replication progression remains unclear, it was suggested that PARP constitutes a signal that switches off DNA synthesis temporarily via the interaction to pol-α, to ensure that lesions are not replicated before DNA repair (Fig. 3).

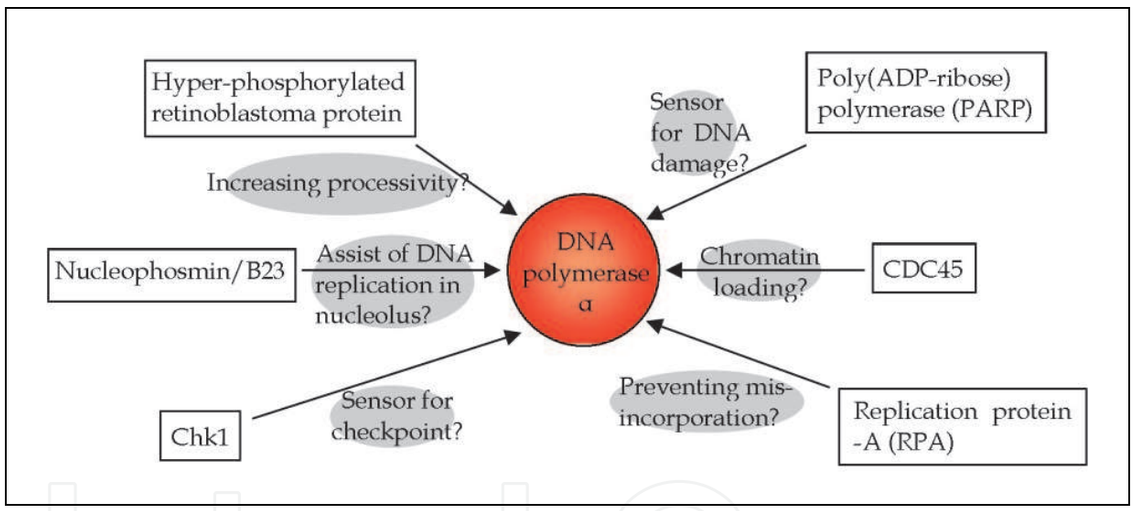

Fig. 3. Pol-α-binding proteins reported.

The author and colleagues found that the nucleophosmin/B23, a major nucleolar protein, is capable of binding to pol-α directly and stimulate its activity (Takemura et al., 1994; Takemura et al., 1999). Nucleophosmin/B23 exists in two isoforms, which are produced through alternative splicing, designed as B23.1 and B23.2. Among them, results demonstrated that purified B23.1 stimulates pol-α activity, and B23.2 does so more weakly than B23.1 (Takemura et al., 1994; Umekawa et al., 2001). Feuerstein et al. have reported that nucleophosmin/B23 is copurified with pol-α on conventional purification (Feuerstein et al., 1990). These results suggest that B23.1 might be involved in DNA replication, aside from the ribosome assembly process in the nucleolus (Orrick et al., 1973; Olson et al., 1974; Schmidt-Zachmann et al., 1987; Chan et al., 1989). Hyper-phosphorylated retinoblastoma protein was reported to bind with pol-α and to raise its processivity (Takemura et al., 1997; Takemura, 2002).

Replication protein-A (RPA) is a single-strand DNA binding protein that is necessary for SV40 DNA replication *in vitro*, stabilizing single-strand DNA after unwinding. RPA comprises three subunits. It reportedly interacts with pol-α. It is known that pol-α pauses at some sites on the natural DNA template. These pause sites are known to be hot spots for nucleotide misincorporation (Fry & Loeb, 1992). RPA reportedly leads pol-α to overcome these error-pone pause sites, suggesting that RPA might reduce misincorporation at some pause sites by pol-α (Suzuki et al., 1994). Maga et al. found that RPA, as an auxiliary factor for pol-α, stabilizes pol-α-primase complex and reduces the misincorporation efficiency of pol-α (Maga et al., 2001).

5. How does pol-α contribute to genomic stability?

Recent studies have revealed various functions of pol-α, not only in RNA/DNA synthesis, but also in the mechanism linking DNA replication to the other cellular regulatory mechanisms. One is the relation between pol-α and mutation, which sometimes causes cancer. Another is the role of pol-α on genomic stability including telomere maintenance.

As described above, pol-α lacks proofreading activity, an intrinsic 3′-5′ exonuclease activity, so it performs DNA synthesis with a fidelity that is lower than the other DNA polymerases pol-δ and pol-ε by ten-fold or more (Pavlov et al., 2006b). Limsirichaikul et al. found that pol-α would leave more mismatched primers without additional extension without proofreading (Limsirichaikul et al., 2003). In one estimation, pol-α is thought to generate 30,000 mutations in one mammalian cell division (Albertson & Preston, 2006). In *Saccharomyces cerevisiae* pol-α, Gly952 residue which is conserved amino acid residue, is necessary for catalytic activity of pol-α, and is therefore important for correct deoxyribonucleotide incorporation (Ogawa et al., 2003; Limsirichaikul et al., 2003). Amino acid substitution (Gly to Ala) of this Gly952 caused the decrease of replication fidelity (Limsirichaikul et al., 2003). On the other hand, amino acid substitution of another amino acid residue, Leu868, of the catalytic subunit of pol-α decreased the replication fidelity of pol-α and increased mutation rates in *Saccharomyces cerevisiae* (Niimi et al., 2004). This mutant, L868F, had a spontaneous error frequency of 3 in 100 nucleotides, and performed 570-fold lower replication fidelity than wild type. Another research group reported that the several mutants including temperature sensitive (ts) mutants of pol-α increases mutation rates (Liu et al., 1999). Recently, Tanaka et al. reported that the base selection step functions to prevent replication errors also in human pol-α and to maintain genomic stability (Tanaka et al., 2010). These results suggest that the replication fidelity of pol-α is important for avoiding mutagenesis and for maintaining genomic integrity.

Various replication stresses engender genomic instabilities. Various mutational analyses of pol-α have shown that the functional aberration affects transcriptional gene silencing, homologous recombination, and chromosomal instability. In *Arabidopsis*, mutation of the catalytic subunit of pol-α reduced histone demethylation in some promoter domains and delayed G_2/M phase with high expression of a G_2/M marker gene, suggesting that pol-α plays a role in some epigenetic states (Liu et al., 2010). Liu et al. also reported that the mutation of pol-α increased the frequency of homologous recombination (Liu et al., 2010). The author and colleagues reported that the inhibition of DNA replication by selective inhibitor of pol-α caused chromosomal instability of cultured opossum (*Didelphis marsupialis virginiana*) cells (Takemura et al., 2011). In *Saccharomyces cerevisiae*, low levels of pol-α gene expression in cells reportedly induced chromosomal translocation (Lemoine et al., 2005) and mitotic and meiotic instability in rRNA gene cluster (Casper et al., 2008), suggesting that pol-α contributes to chromosomal stability throughout the cell cycle directly in some mechanism.

Telomere maintenance is an important task that must be done to ensure chromosome stability, especially in germ line and cancer cells. Using pol-α-ts mutant, tsFT20 (Murakami et al., 1985), results showed that the telomere G-tail was induced to increase markedly in the overall telomere length when pol-α was inactivated (Nakamura et al., 2005). It is particularly interesting that the inhibition of pol-α also caused a considerable increase of Robertsonian chromosome fusions (Nakamura et al., 2005). These observations correspond to our *in vitro* data indicating that pol-α itself performs replication slippage on a telomeric repeat (Nozawa et al., 2000), and that hyper-phosphorylated retinoblastoma protein suppresses replication slippage by pol-α in normal cells (Takemura et al., 2008). These results suggest that the steady state expression of pol-α and its normal regulation by other accessory factors suppresses chromosome abnormality, including the expansion of telomeric repeat.
In telomeric DNA replication, Cdc13 protein is known to be a key factor for replication and telomere capping in yeast (Evans & Lundblad, 2000). Cdc13 is also known to interact with the catalytic subunit of pol-α (Qi & Zakian, 2000), suggesting that pol-α has an important role in telomere DNA replication. Grossi et al. described that the second-largest subunit of pol-α physically interacts with Stn1 protein, which is known to be the regulator of telomere end (Grossi et al., 2004). In pol-α (*cdc17/pol1*), a mutant of yeast revealed defects in telomeric lagging strand synthesis (Martin et al., 2000). These results suggest that pol-α acts as a regulator of telomeric lagging strand synthesis completion linking to the action of telomerase, via the interaction with Cdc13 and Stn1.

6. Remaining mysteries of pol-α

To date, much research about one eukaryotic key DNA polymerase, pol-α, has been performed by numerous research groups. The initiation mechanism of pol-α has been well studied, and the interaction of pol-α with other DNA polymerases and other replication factors has been clarified. In addition to these previous efforts by previous researchers, several studies have revealed unknown profiles of pol-α.

6.1 Detailed structure, attached molecules, and intracellular amounts of pol-α

It was reported that purified pol-α from calf thymus showed microheterogeneity in its catalytic subunit, especially in their molecular weights: 150 kDa, 145 kDa, and 140 kDa (Masaki et al., 1982). Pol-α, pol-δ, and pol-ε of rat ascites hepatoma cells, Novikoff hepatoma cells, were shown to differ from those of normal rat liver cells in their K_m values of DNA-binding (Popanda et al., 1995). It has not been clarified how these fluctuations about molecular 'species' of pol-α have been revealed, in spite of the suggestion that the 'programmed proteolysis' of the catalytic subunit of pol-α might be performed (Masaki et al., 1982).
Simbulan et al. reported that sialic acid-containing glycolipids inhibit the activity of pol-α (Simbulan et al., 1994). Although sialic acid is an extremely important carbohydrate attached to the carbohydrate chain, such as that on cell membrane, it is not known whether it functions in the cell nucleus. Reportedly, pol-α itself is one such glycoprotein (Bhattacharya et al., 1979; Hsi et al., 1990). Detailed roles of the carbohydrate chain on pol-α have not yet been clarified.
One more mystery of pol-α is why it is abundantly present in mammalian cells. Pol-α has been known to have large quantities in the cell nucleus compared with other pol-δ and pol-ε. Among them, only pol-α molecules required for DNA replication initiation are thought to

participate in DNA replication machinery. The reasons for the large number of pol-α molecules apparently standing by in the cell nucleus remain unknown.

6.2 How has pol-α evolved?

It has been proposed that three DNA polymerases–– pol-α, pol-δ, and pol-ε––have evolved from ancestral ε-like DNA polymerase (Edgell et al., 1998). Molecular evolutionary studies suggest that pol-ε is an ancestral type of these replicative DNA polymerases in eukaryotes (Edgell et al., 1998). Actually, pol-α and pol-δ were thought to have arisen from two gene duplications, emerging from ancestral pol-ε (Edgell et al. 1998). Filée et al. suggested that the evolutionary history of DNA replication proteins such as DNA polymerases involve significant exchanges among viruses, plasmids, and their host cells (Filée et al., 2002). According to the molecular evolutionary analysis, the author advanced the hypothesis that ancestors of pol-α gene were derived from a DNA virus-like organism, which had some eukaryote-like characteristics, such as poxviruses (Takemura, 2001). Villarreal & DeFilippis proposed that the origin of the eukaryotic pol-δ should be some algal viral DNA polymerases (Villarreal & DeFilippis, 2000). In spite of these attractive studies, the mystery of the evolution of the eukaryotic DNA polymerases remains to be elucidated.

6.3 How has pol-α contributed to eukaryotic evolution?

In mammals, a gene of catalytic subunit of pol-α is established in the X-added region of an X chromosome in eutherians, although it is on the autosome in metatherians (marsupials). This locational property is thought to have arisen from chromosomal translocation that occurred in the eutherian lineage 105 million years ago (Fig. 4). One large segment of one autosome was thought to translocate to X chromosomes: the so-called 'X-added' region (Graves, 1995). Although the effects of this large-scale translocation on the evolution of eutheria are entirely unknown, according to the simulation of spontaneous mutation rates in these two mammalian groups, the author has proposed that the X-linked pol-α gene contributes to greater diversity of eutherian mammals (Takemura, 2008). The solution of this problem, however, will require much more time and study.

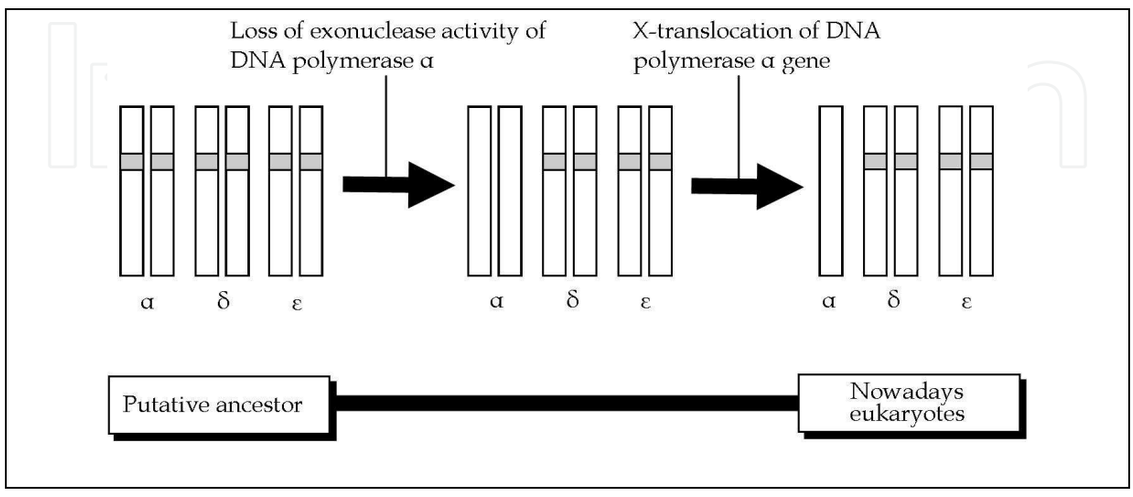

Fig. 4. Remaining mystery of pol-α.

7. Acknowledgements

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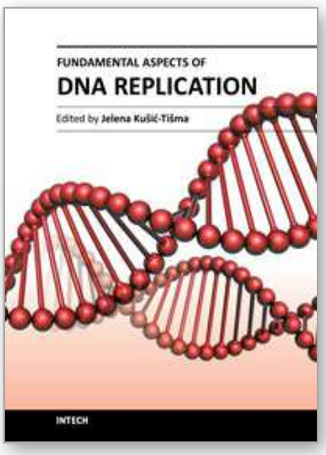

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DNA replication, the process of copying one double stranded DNA molecule to produce two identical copies, is at the heart of cell proliferation. This book highlights new insights into the replication process in eukaryotes, from the assembly of pre-replication complex and features of DNA replication origins, through polymerization mechanisms, to propagation of epigenetic states. It also covers cell cycle control of replication initiation and includes the latest on mechanisms of replication in prokaryotes. The association between genome replication and transcription is also addressed. We hope that readers will find this book interesting, helpful and inspiring.

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