Review Letter

# DNA POLYMERASES OF EUKARYOTES

A. M. HOLMES and I. R. JOHNSTON
*Biochemistry Department, University College London, Gower Street, London WC1E 6BT UK*

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## 1. Introduction

Although the currently received view of the enzymology of DNA synthesis in prokaryotes is based on a substantial body of information and controversy, it is usually overlooked that serious interest in the DNA polymerases (EC 2.7.7.7.) of both prokaryotes and eukaryotes commenced in the same period, 1956–8. In the following review, a brief summary is given of the situation in prokaryotes before consideration of that in eukaryotes.

## 2. Prokaryote DNA polymerases

Since the DNA polymerase isolated by Kornberg and his colleagés from *E. coli* [1] (now referred to as DNA polymerase I) held prominence for more than a decade, a central rôle for it in replication was often implicitly assumed. The discovery in 1969 by DeLucia and Cairns of a mutant retaining only 0.5–1% of assayable DNA polymerase [2] but still capable of DNA replication, led to increased speculation concerning the true function of DNA polymerase I, which at that time was the only well characterised DNA polymerase of *E. coli*. In the event, the major outcome of the discovery of the DeLucia and Cairns mutant, which did not definitively eliminate DNA polymerase I from a replicative rôle, was the impetus it gave to the search for new DNA polymerases. As a result, two further DNA polymerases were isolated, purified and characterised, DNA polymerases II [3–5] and III [5–7] (see table 1). While polymerase II is believed to be a repair enzyme

Table 1
*E. coli* DNA polymerases

| DNA Polymerase | I | II | III |
|---|---|---|---|
| Mol. wt × $10^{-3}$ | 109[17] | 90[15]; 120[16] | 140[6,7,12]; 180[17] |
| Preferred template | Activated DNA | Gapped DNA | Gapped DNA |
| No. of molecules/cell | 400 | 20 | 10 |
| In vitro rate of elongation (nucleotides/minute/molecule of DNA polymerase) | 1000 | 300 | 15 000 (In vivo 100 000 nucleotides per minute per replicating fork). |
| Nucleases | 5′ → 3′<br>3′ → 5′ | 3′ → 5′ | 5′ → 3′<br>3′ → 5′ |
| Thiol group for activity | No | Yes | Yes |

[8], polymerase III has been shown to be the product of the dnaE gene [9,10], temperature sensitive mutants in which cease replication immediately on transfer to the non-permissive temperature. Reconstruction experiments also indicate that this polymerase is directly involved in the replication process [11]. Although disagreement exists as to whether DNA polymerase III contains one sub-unit of 140 000 mol. wt. [7,12] or two of 90 000 [13], nevertheless two further forms of it have been described, namely DNA polymerase III star [13] and holoenzyme DNA polymerase III containing the initiation factor, copol III star [14]. The precise rôle of these species in replication will probably become clearer when confusion over the subunit structure is resolved (table 1).

*Bacillus subtilis* also contains three DNA polymerases. DNA polymerase I may differ from the analagous *E. coli* enzyme in the absence of $3' \rightarrow 5'$ and $5' \rightarrow 3'$ exonuclease activities [17]. DNA polymerase III of *B. subtilis* is unique in that it is inhibited in in vitro experiments by 6-(*p*-hydroxyphenylhydrazino)-uracil, although the other DNA polymerases of *B. subtilis* and all DNA polymerases of *E. coli* are not affected [18,19]. In vivo studies show that 6-(*p*-hydroxyphenylazo)-uracil inhibits DNA replication, but not repair processes [20]. A spontaneous mutant, resistant to the drug in vivo has been shown to have a resistant DNA polymerase III [21,22]. Confirmation of the rôle of DNA polymerase III in replication in *B. subtilis* has come from the isolation of temperature sensitive DNA polymerase III mutants which are unable to replicate DNA at the restrictive temperature [22,23].

The DNA polymerase isolated from *Micrococcus luteus* has properties identical to those of *E. coli* DNA polymerase I [17]. At present there is no reason to doubt that many if not all bacterial species will possess this fundamental group of polymerases.

## 3. Eukaryote DNA polymerases

In eukaryotes a system capable of in vitro DNA synthesis was first described shortly after that in *E. coli* [24]. Subsequently most preparations of DNA polymerase were isolated and partially purified from the high speed supernatant fraction of cell and tissue homogenates (reviewed in [25,26]). In 1971 several laboratories distinguished and partially characterised a further DNA polymerase located predominantly in the nucleus [27–31]. Subsequently four main classes of DNA polymerases have been defined in mammalian systems, namely DNA polymerases -$\alpha$, -$\beta$, -$\gamma$ and mitochondrial (mt) [32] (table 2).

### 3.1. *DNA polymerase-α*

This is a high mol. wt. enzyme usually isolated from the soluble fraction of cell and tissue homogenates. It shows high activity with activated double-stranded DNA but will not use the initiated homopolymer complex poly (A).oligo (dT) $_{\overline{15}}$ [33,34]. It will, however, use oligoribonucleotide initiated polydeoxynucleotides [33–35] and RNA-primed DNA [36]. It is sensitive to *N*-ethylmaleimide indicating a sulphydryl requirement for enzyme activity [37,38]. At physiological ionic strengths and higher the $\alpha$-polymerase sediments at 6–8S [39,40] whereas at ionic strengths below 0.07 it aggregates to greater than 10.7S [40–42].

Circumstantial evidence has implicated this enzyme in DNA replication. Levels of extractable activity can be correlated with in vivo rates of DNA replication in regenerating rat liver [43,44], mouse L cells [45] HeLa [46], BHK cells [47] as well as in chemically induced tumours [26] and phytohaemagglutinin stimulated lymphocytes [37].

While the $\alpha$-polymerase is routinely isolated from the soluble fraction, under certain conditions a substantial part of the activity (up to 40%) can be found in the nucleus [27,48]. In non-aqueous isolation procedures where cells were freeze-dried and homogenised in spectral grade glycerol, 90 per cent of the $\alpha$-polymerase was found in the nucleus [49]. Nuclei extruded from cells by the action of cytochalasin B also contain greater than 90% of the $\alpha$-polymerase [50]

Studies on the DNA polymerase of sea urchins have suggested that polymerase is translocated into the nucleus during S phase [51]. The DNA polymerase level of unfertilised sea urchin eggs is high and the amount of activity per embryo does not change during early development. As development proceeds the activity in the nuclear fraction increases and that of the cytoplasm decreases. (After fertilisation only about 2% of the total protein of sea urchin embryos undergoes turnover in the first 24 h. DNA polymerase does not turnover any faster than other proteins and

Table 2
Mammalian DNA polymerases

| DNA Polymerase | α | β | γ | mt |
|---|---|---|---|---|
| Sedimentation coefficient[a] | 6–8S | 3.3S | 6.1–6.3S | 8–9S |
| Location[b] | mainly cytoplasmic | mainly nuclear | both cytoplasmic and nuclear[d] | mitochondria |
| Thio group for activity[c] | Yes | No | Yes | No |
| Nuclease | None | None | ? | Yes[e] |
| Template utilisation | | | | |
| Activated DNA | + | + | + | + |
| poly (A). oligo $(dT)_{\overline{15}}$ | – | ++ | ++ | – |
| poly (dT). oligo $(rA)_{10}$ | ++ | – | ++ | – |

[a] Measured under disaggregating conditions (ionic strength > 0.15)
[b] As routinely extracted using aqueous solutions.
[c] Measured by requirement for reduced thiols in the assay and/or inhibition by thiol reagents.
[d] Two cytoplasmic enzymes, one of which appears to be the same as the nuclear.
[e] Relatively crude preparations.

little is synthesised [52]). When nuclei were isolated in aqueous media from synchronously dividing embryos (i.e. shortly after fertilisation) at different points in the cell cycle, it was found that DNA polymerase became associated with the nucleus at the very beginning of S phase, the activity per mg. of nuclear protein rising ten-fold and then falling later in S phase, but not to the level of the previous cycle. The increment remaining probably accounts for the progressive rise in nuclear specific activity as development proceeds. This pattern of decrease and increase is unique to DNA polymerase. Deoxyribonuclease, thymidine kinase and lactate dehydrogenase did not show the same consistent pattern of change [53].

In view of the points discussed above these results may also be interpreted by saying that DNA polymerase is bound in the nucleus throughout but that at the beginning of S phase it is more tightly bound and much less susceptible to leaching out in aqueous media compared with other parts of the cell cycle.

Although DNA polymerase-α was first observed in 1957 [24] it has not yet been purified to homogeneity. Heterogeneity of the enzyme activity has been observed in a variety of tissues and cells such as calf thymus [34,54,55], rat liver [34], mouse myeloma [56,57] BHK cells [58] and rabbit bone marrow [59]. This heterogeneity may be due to aggregation [26], proteolysis or possibly to association with other proteins of the replication complex and these probably account for some of the difficulties in purification. An additional problem is the low level of enzyme (about 1 mg per kg tissue) even in tissues synthesising DNA at a high rate [60].

When partially purified calf thymus DNA polymerase-α was fractionated on DEAE-cellulose with a 30–250 mM potassium phosphate gradient at pH 7.8, several peaks of activity were resolved when assays were carried out with activated DNA. In order of elution these were enzymes A (sedimenting at 8S in glycerol gradients of 0.5 ionic strength) eluting between 55–95 mM, enzyme B (5.2S) eluting between 95–110 mM and enzyme C (7.3S) eluting between 125–160 mM phosphate. Preliminary estimates of molecular weight gave values of 200–230 000, 100–110 000 and 155–170 000, respectively, for A, B and C [34]. However, the relative levels of these three enzymes

were found to vary from one preparation to another and a more frequently observed elution profile on DEAE-cellulose is one in which the major species are two A enzymes, $A_1$ and $A_2$, eluting between 55–75 mM and 75–95 mM phosphate respectively, and enzyme C [54,61]. The levels of the 5.2S enzyme are now considerably reduced and, since this species has been observed to arise from the 7.3S enzyme during further purification (unpublished work, AMH), it may be a proteolytic breakdown product (cf. [45]).

If the standard DEAE-cellulose profile is assayed at pH 7.0 using poly (dA). $(dT)_{10}$ (20 : 1 base ratio) as template instead of with activated DNA, the incorporation of [$^3$H] dTMP is greatest in a peak eluting between 110–125 mM phosphate, which does not correspond to any of the activated DNA dependent peaks, $A_1$, $A_2$, B or C. This activity provisionally designated enzyme D, sediments on standard glycerol gradients at 6.6–7.0S. Using a $D_{20,w}$ estimate from a calibrated Sepharose 6-B column [34] gives a molecular weight of 140–150 000. Although all species of calf thymus α-polymerase will use the synthetic template-initiated complex, the relative preference for poly (dA). $(dT)_{10}$ versus activated DNA observed for enzyme D at pH 7.0 is the reverse of that for $A_1$ or $A_2$. However, these differences are pH dependent and largely disappear if assays are done at pH 6.5 which is near the optimum for $A_1$ and $A_2$ on poly (dA). $(dT)_{10}$ [62].

Early attempts to ascertain the sub-unit structure of the α-polymerase using dodecylsulphate polyacrylamide gel electrophoresis indicated that a polypeptide of 54 000 might be an important component [34]. However, using enzyme preparations of specific activity one to two orders of magnitude higher than those used previously, a polypeptide of 150–160 000 (determined on SDS gels) appears to correlate with both $A_1$ and $A_2$ enzyme activity profiles on normal polyacrylamide gel electrophoresis [63]. When 2.4 M urea was used to convert enzymes $A_1$ and $A_2$ into C (mol. wt. 155–170 000) the C enzyme produced, again showed a main band at 150–160 000 on SDS gels which could be correlated with enzyme activity. Since the A enzymes did not show polypeptides on SDS gels corresponding to their molecular weight (200–230 000), the results above suggest that urea is removing a sub-unit(s) or fragment of 50–70 000 mol. wt. from them, leaving the polymerase sub-unit of 150–160 000 as enzyme C. The possibility that the removal of the 50–70 000 mol. wt. portion is due to the proteolysis of a 200–230 000 polypeptide on exposure to 2.4 M urea is possible though unlikely, since in $A_1$ or $A_2$ not exposed to urea, no polypeptide of this size appears to exist. The laboratories of F. J. Bollum [60] and D. Korn [64] have also produced evidence that DNA polymerase-α activity resides in a polypeptide of 140–160 000 in calf thymus and human KB cells, respectively.

While it might be expected that the synthesis of a complementary polydeoxynucleotide chain on an initiated template would involve the direct procession of a polymerase molecule along the template, recent evidence indicates that DNA polymerases-α and β as well as *E. coli* DNA polymerase I are distributive in in vitro systems containing no other components of the replication complex. Thus the enzyme only elongates the initiator by a small number of bases before dissociating from it [65,66]. Association with other proteins of the replication apparatus in vivo would be expected to reduce the frequency of polymerase dissociation from the template as observed in the reconstructed system for $T_4$ [67].

### 3.2. *DNA polymerase-β*

The low mol. wt DNA polymerase of mammalian tissues, DNA polymerase-β is characteristically found in nuclei. Although varying proportions of it are also found in the cytoplasm, the precise distribution between nucleus and cytoplasm may be a function of nuclear stability during isolation procedures (i.e. of levels of divalent cations used in aqueous isolation media) as well as of the ratio of nuclear to cytoplasmic volume [26]. The absolute amounts of polymerase-β activity remain fairly constant throughout a range of physiological states in various tissues at about 100 nmol total deoxyribonucleotide incorporated/h/g of tissue in a standard polymerase assay using activated DNA. Thus in proliferating tissues, it represents 5–10% of the total polymerase activity but about 50% in non-proliferating cells and tissues. This suggests that it may play no direct role in replication [46,47].

Results from several laboratories indicate that it is a single polypeptide of mol. wt. 42 000–45 000 [68–70] although in chick embryo two β-polymerases have been claimed of mol. wts. 27 000 and 54 000 based on a 27 000 mol. wt. sub-unit [71].

DNA polymerase-β from different species sediments at 3.3–3.5S at physiological ionic strengths or greater [59,71,42]. It binds tightly to DNA-cellulose columns unlike polymerase-α [31,72]. Activated double-stranded DNA and poly (A). oligo $(dT)_{\overline{15}}$ are equally good templates [59,73,74] but poly(dT).oligo (A) is not used to any great extent for dAMP incorporation [33,62] and it will not initiate on an heteropolymeric RNA initiator [36]. Unlike polymerase-α, polymerase-β is insensitive to *N*-ethylmaleimide [37,38]. It has an alkaline pH optimum [38,48,59,75] and an isoelectric point of 9.2 [48,76]. As with polymerase-α, no associated nuclease activities have been found [69,75, 77]. Some reports show the partially purified enzyme to contain NDP kinase activity in common with many other DNA polymerases [48,75,78]; it may however simply be a persistent contaminant. Unlike polymerase-α, polymerase-β is not stimulated by DNA unwinding proteins [79].

DNA polymerase-β is immunologically distinct from polymerase-α. This was shown using antibody raised in rabbits against partially-purified polymerase-α from HeLa cells [42], reversing an earlier claim by Chang and Bollum [80]. The same HeLa DNA polymerase-α antiserum also partially inhibited the DNA polymerase-α of Chinese Hamster cells suggesting that common peptide sequences might exist in polymerase-α from different species [42]. More recently the absence of any relationship between α- and β-polymerases was confirmed, using antiserum to DNA polymerase-α raised in rats, in both direct neutralisation assays, and additionally by immunoprecipitation of the polymerase-α–antibody complex with goat-antirat immunoglobulin [81]. The same study revealed that the reverse transcriptase of five RNA tumour viruses were immunologically distinct from polymerase-α. More recent work also shows the α- and β-polymerases of chick embryo to be immunologically unrelated [81a].

The precise function of DNA polymerase-β is not yet known.

### 3.3. *DNA polymerase-γ*

This enzyme comprises only about 1% of the total cellular DNA polymerase activity [46]. It has been less well studied than DNA polymerases-α or -β and its purification will no doubt prove a difficult undertaking because of the low levels. It has been found in HeLa cells [82], human lymphoblastoid cells [83], calf thymus [70,84] and mouse myeloma [56]. Although its level is doubled within two hours of the start of S phase in HeLa cells, it is not known what role if any it plays in replication. Two cytoplasmic γ-polymerases, I and II have been found in HeLa and one of these (II) appears to be similar to the nuclear γ-polymerase [85], γ-polymerase I copies only poly (A) among homoribopolymers, but II is able to copy poly (C), poly (U) or poly (I) in addition. Both sediment at 6.1–6.3 S [86] giving an apparent mol. wt of 110 000. $K_M$ values for deoxynucleoside triphosphates ($5 \times 10^{-7}$ M) are one order of magnitude lower than those for HeLa polymerases-α or -β suggesting that these triphosphates might be readily utilised by γ-polymerases early in S phase when their pool sizes are relatively low [85].

γ-polymerase requires thiol groups for maximal activity [87], this fact distinguishing it from polymerase-β. The preferred template of γ-polymerase II is poly (A).$(dT)_{\overline{15}}$ which it uses three times better than activated DNA, whereas γ-polymerase I uses these templates equally well. Although DNA polymerases -β and -γ, unlike polymerase-α, will both use poly (A).$(dT)_{\overline{15}}$ neither will use poly (C).$(dG)_{\overline{15}}$ or natural RNA to any appreciable extent unlike reverse transcriptase [85,86].

HeLa DNA polymerases-γ I and II are not inhibited by antibodies prepared against the reverse transcriptase from Mason-Pfizer monkey virus, Woolly monkey virus or the Rauscher Murine leukaemia virus [85]. The corresponding enzymes from human lymphocytes are not inhibited by antibody prepared against simian sarcoma virus reverse transcriptase [86,88]. Neither are DNA polymerases-α and -β inhibited by antibody against primate type C viral reverse transcriptase [88,89].

The conclusion therefore is that DNA polymerases-α, -β and -γ are distinct from each other and from viral reverse transcriptase.

### 3.4. *Mitochondrial DNA polymerase*

This enzyme is located only in the mitochondria. It has been partially purified and as yet is incompletely characterised [90–93]. Mitochondrial DNA polymerases from various sources exhibit differences in their template preferences [90–92], apparent molecular weights [92,94] and salt optima [91,93]. These differences may be explicable by varying purity

of the enzymes, contaminating nucleases [91,92,95] or the existence of more than one species of enzyme [95].

DNA polymerase-mt resembles α-polymerase in its inability to copy oligodeoxyribonucleotide initiated homoribopolymers such as $(dT)_{12-18}$.poly (A), $(dA)_{12-18}$.poly (U), $(dG)_{12-18}$.poly(C) and $(dC)_{12-18}$.poly (I), all of which are copied by eukaryotic γ-polymerases [85,92].

Three additional features distinguish DNA polymerase-mt. Its relative insensitivity to *N*-ethylmaleimide distinguishes it from DNA polymerase-α but not from β; it is unable to use $(dG)_{12}$.poly (dC) unlike both α- and β-polymerases which use this complex with high efficiency; finally, it is exceptionally heat labile ($t_{\frac{1}{2}}$ at 45°C < 5 min) compared with α- and β-polymerases [92].

The mitochondrial DNA polymerase sediments at high ionic strength at 8–9S [94] indicating an apparent mol. wt. of 159 000 although values of 100 000 and 106 000 have also been reported [96,92]. When partially purified DNA polymerase-mt acts on intact mitochondrial DNA the product resembles intermediates usually observed in vivo [95]. Although, as mentioned earlier certain mitochondrial DNA polymerase preparations contain an endonuclease, the role of this, if any, in mitochondrial DNA replication remains to be established. However, the occurrence of an integral nuclease in mitochondrial DNA polymerase would not be altogether surprising in view of the alleged endosymbiotic origins of these organelles.

### 3.5. *Terminal transferase*

In addition to the four deoxyribonucleoside triphosphates and a divalent metal, the enzymes described in previous sections also require a template and initiator for activity. By way of contrast a further deoxyribonucleotidyl transferase activity, discovered initially in calf thymus, does not require a template and utilises any one of the four deoxyribonucleoside triphosphates to extend an initiator from the 3′-hydroxyl end [97]. The initiator may be single stranded DNA or a short (⩾ 3) oligodeoxynucleotide [98]. The presence of all four deoxyribonucleoside triphosphates inhibits this terminal transferase activity. The calf thymus enzyme has been purified to homogeneity and its properties described [99]. It has a mol. wt. of about 33 000 consisting of two sub-units of approximately 26 000, and 8000 mol. wt. It has an unusually low partial specific volume (0.65 ml/g) perhaps due to the presence of zinc in the molecule [100]. (This observation and that of the presence of $Zn^{++}$ in *E. coli* DNA polymerase I [101], sea urchin DNA polymerase [102] and AMV reverse transcriptase [103,103a] raises the possibility that all DNA polymerases are zinc-containing enzymes). Earlier evidence indicated that the terminal transferase was unique to the thymus [104]. More recent work has identified terminal transferase in peripheral lymphocytes of patients with acute lymphoblastic leukaemia [105] acute myelogenous leukaemia [106], chronic myelogenous leukaemia [106, 107] and particularly high levels (14–16 units/$10^8$ cells) were found in the bone marrow and peripheral blood of a patient with a poorly differentiated acute myelomonocytic leukaemia [106]. It is also present although in very low levels, in bone marrow (0.7 units/$10^8$ cells) and lymphocytes (0.01 unit/$10^8$ cells) of normals. These observations suggest therefore that terminal transferase is present in lymphoid progenitor cells from bone marrow as well as thymocytes. The function of the enzyme remains obscure but it has been postulated that it might be involved in the immune response by diversifying the section of the genome coding for the variable regions of the immunoglobulin chains [108].

### 3.6. *Viral DNA polymerases*

Further types of DNA polymerase can be extracted from eukaryotic cells infected with viruses. By far the best known is that class of polymerases associated with RNA tumour viruses (more recently reviewed in [109 and 110]). Since they are able to make a DNA copy of the viral RNA genome they are referred to as reverse transcriptases (RNA-dependent DNA polymerases). The enzyme, which in avian myeloblastosis virus comprises about 3% of the virion protein (equivalent to 70 molecules enzyme per virion [111]), is associated with the viral core [112, 113]. The biological role of reverse transcriptase is indicated by the fact that mutants of Rous sarcoma virus, temperature sensitive in the initiation of both viral replication and host cell transformation, also possess thermolabile reverse transcriptase activities [114]. One current view of the mechanism of conversion of the 70S viral RNA to double-stranded DNA which is ultimately integrated into the host genome, involves the initial formation of an RNA:DNA hybrid followed

by removal of the viral RNA strand and its replacement by the second DNA strand in a DNA-dependent step [109]. Both RNA- and DNA-dependent steps may be catalysed by reverse transcriptase since the activities on exogenous RNA and DNA templates are inseparable on purification of DNA polymerase activity and are heat inactivated at the same rate (see for example [115]). Further, a nuclease, ribonuclease H, which will degrade the RNA strand of an RNA:DNA hybrid has been found to be associated with reverse transcriptase. The studies on Rous sarcoma virus temperature sensitive mutants referred to earlier showed RNA-dependent DNA polymerase, DNA-dependent DNA polymerase and RNase H activities to be equally heat labile compared with the wild type activities, suggesting that all three activities reside in the same molecule [114]. The evidence that RNAse H activity is a function of the mammalian reverse transcriptase has been questioned [116] but more recent data support the close relationship of these activities [117]. Unlike most cellular RNAse H's the viral enzyme is exonucleotytic in action [118].

Reverse transcriptase transcribes 70S viral RNA very efficiently if given an initiator RNA. It is now thought that in the intact virion, non-acylated tryptophan tRNA of host cell origin acts as the initiator [119,120], although some evidence indicates longer RNA initiators may be involved [121] (a finding consistent with the Cooper and Wyke replication model [122]). The small size of the viral DNA product made in vitro (5–6S) is probably a function both of the assay conditions used since optimising these has allowed much larger DNA products to be synthesised including a small but significant amount of full length transcripts [123], and of the absence of ligating enzymes.

Although the enzymatic activities of avian and mammalian reverse transcriptases appear to be the same, they are structurally dissimilar. For instance, Rauscher murine leukaemia virus has been reported to have a molecular weight of 70 000 [124], that of Rous sarcoma virus 110 000 [125] while intracellular gibbon ape leukaemia viral enzyme exists in two forms 70 000 and 130–140 000 [126]. Avian myeloblastosis virus reverse transcriptase has been isolated with one and with two subunits, $\alpha$ of 69 000 mol. wt. and $\beta$ of 110 000 [127]. Both $\alpha$ and $\alpha\beta$ forms exhibit reverse transcriptase and RNase H activity in vitro [128]. $\beta$ has been shown to give rise to $\alpha$ by proteolytic cleavage [129,130]. Both sub-units contain the reverse transcriptase and RNAse H activity [129]. It is not yet known which is the active form in vivo. The 130–140 000 mol. wt. form of the gibbon ape leukaemia virus enzyme appears to arise by aggregation of a 70 000 form (which is the only one present in the mature virion) with some host cell protein which alters its template and antigenic properties [126].

Reverse transcriptases have been isolated from various human acute leukaemic blood cells [88,126, 131]. Human leukaemia reverse transcriptase has been demonstrated to be immunologically related to primate type C viral reverse transcriptases, but not to other type C viral reverse transcriptases or the avian myeloblastosis viral enzyme [88,89]. There is as yet no evidence showing the existence of reverse transcriptase in uninfected normal mammalian cells [132].

The second type of viral DNA polymerase to be found in eukaryotic cells are those induced by the DNA viruses. These DNA-dependent DNA polymerases, none of which has been extensively purified, are a diverse group.

After infection of BHK or human epithelioid carcinoma cells by *Herpes simplex* virus, a new DNA polymerase, with properties which distinguish it from the cellular DNA polymerases, was observed in both nuclei and cytoplasm [133,134]. The enzyme has been partially purified and its properties discussed [135]. It is large (mol. wt. 180 000) and requires salt and thiol groups for maximal activity. Although a nuclease copurifies with the DNA polymerase, as the enzyme is not homogeneous, it is difficult to say with certainty whether or not it is an integral part of the molecule. A nuclease also copurifies with the DNA polymerase isolated from cells infected with vaccinia [136]. These two DNA polymerases, and also that from the herpes virus-induced Marek's disease [137], can be distinguished from each other on the basis of size, template properties and chromatographic properties [32].

### 3.7. *Other eukaryote DNA polymerases*

In plants and lower eukaryotes the numbers and types of DNA polymerases have been less extensively studied. Two related high mol. wt. enzymes, distinct from the mitochondrial and chloroplast species, have been described in *Euglena gracilis;* one of these pol A

is predominantly nuclear and the other pol B predominantly cytoplasmic. On entry into exponential growth pol B activity increases to a greater extent than pol A [138,139] (These references also include some discussion of lower eukaryote DNA polymerases.) In yeast (*Saccharomyces cerevisiae*) two high mol. wt. (8S) non-mitochondrial DNA polymerases have also been isolated and partially purified [140,141]. Several properties of both enzymes bear a resemblance to those of mammalian DNA polymerase-α [141]. No 3.4S enzyme (c.f. mammalian DNA polymerase-β) has been detected [142]. In *Tetrahymena pyriformis* the major DNA polymerase activity from exponentially growing cells has been purified 12 000-fold. At ionic strengths greater than 0.25 its mol. wt is 80 000 [143]. In the presence of $Mn^{++}$ it can use a polyribonucleotide template [144]. All the above polymerases (*Euglena, Saccharomyces* and *Tetrahymena*) are inhibited by thiol reagents.

In the true slime mould, *Physarum polycephalum,* and the cellular slime mould, *Dictyostelium discoideum,* only a high mol. wt. DNA polymerase, with properties resembling mammalian DNA polymerase-α has been observed [145,146]. The enzyme sediments at 10S in low salt and 7S in high salt gradients and is sensitive to *N*-ethyl maleimide [146]. Only one DNA polymerase has been described in the smut fungus, *Ustilago maydis,* (mol. wt. 100–110 000) [147]. This enzyme is the only eukaryotic DNA polymerase, so far, which has been genetically implicated in DNA replication, a temperature sensitive mutant in DNA replication having a temperature sensitive DNA polymerase [148].

Sea urchin DNA polymerase has been extensively purified and shown to have a mol. wt of 150 000. The purified enzyme appears to be devoid of associated nuclease activity [149].

It would appear that only in higher eukaryotes, both plant and animal, that a need for different types of DNA polymerases in the cell has arisen. Although we can tentatively assign a role in replication to DNA polymerase-α and DNA polymerase-mt, the true functions of DNA polymerases-β and -γ are unknown. Lower eukaryotes would appear to be devoid of β and γ, the functions of which may be carried out by their major DNA polymerases. However, other DNA polymerases could be present in such small amounts that they have yet to be detected. In this context it is interesting to note that a further species of DNA polymerase, present in extremely low amounts, has recently been isolated from the nuclei of KB cells [150]. There is, as yet, no evidence of any structural similarity between prokaryotic and eukaryotic DNA polymerases despite their identity of function.

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