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Isolation and sequence determination of the cDNA encoding DNA polymerase δ from *Drosophila melanogaster*

(DNA replication; PCNA; cloning; PCR; antibody; sequence conservation)

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SUMMARY

The cDNA encoding the catalytic subunit of *Drosophila melanogaster* (*Dm*) DNA polymerase δ (Pol δ) was isolated by a combination of PCR amplification and cDNA library screening. The cDNA is 3457 nucleotides in length and contains an open reading frame (ORF) that encodes a protein of 1092 amino acids (124 799 Da). The ORF contains the sequence that was determined for a peptide from the purified catalytic subunit of *Dm* Pol δ . Polyclonal antibodies raised against *Dm* Pol δ specifically recognize a protein of the expected size when the cDNA is expressed in either *Escherichia coli* or insect cells. Comparison of the deduced aa sequence with other Pol δ sequences demonstrates that Pol δ is one of the most highly conserved of the DNA polymerases.

INTRODUCTION

Three DNA polymerases, DNA polymerase α , δ and ϵ (Pol α , δ and ϵ) are required to replicate eukaryotic chromosomal DNA (for review, see Wang, 1991; Stillman, 1994). Pol δ and ϵ also appear to be involved in DNA repair (Nishida et al., 1988; Budd and Campbell, 1995; Aboussekhra et al., 1995). Recent studies that link mutations of Pol δ to the replication error (RER) phenotype

of some colorectal tumors are in agreement with this idea (da Costa et al., 1995).

Pol δ is highly conserved both structurally and functionally. It consists of two subunits of approx. 120 and 50 kDa. The 120-kDa subunit has the polymerase and 3'-5' exonuclease activities associated with the two subunit enzyme. The function of the 50-kDa subunit is unknown. Deoxynucleotide polymerization by Pol δ is distributive; however, it becomes highly processive in the presence of the accessory protein, proliferating cell nuclear antigen (PCNA). The strong functional conservation of Pol δ is supported by the finding that calf thymus PCNA stimulates the processivity of Pol δ from *S. cerevisiae* and *S. cerevisiae* PCNA stimulates the processivity of the calf thymus Pol δ (Bauer and Burgers, 1988). Pol δ from *S. cerevisiae* can also substitute for human Pol δ in SV40 replication in vitro (Tsurimoto et al., 1990).

We have been using *Drosophila melanogaster* (*Dm*) embryos as a model for the study of eukaryotic DNA replication and repair (Lehman and Kaguni, 1989). As part of this study, we purified Pol δ from 0–2-h embryos to near homogeneity and demonstrated that it consists of only a single 120-kDa polypeptide, that is unresponsive

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Abbreviations: aa, amino acid(s); Ab, antibody(ies); bp, base pair(s); cDNA, DNA complementary to RNA; *Dm*, *Drosophila melanogaster*; Exo, exonuclease; HSV-1, herpes simplex virus type 1; kb, kilobase(s) or 1000 bp; nt, nucleotide(s); ORF, open reading frame; PCNA, proliferating cell nuclear antigen; PCR, polymerase chain reaction; Pol, DNA polymerase; *Pol*, gene (DNA) encoding Pol; *S.*, *Saccharomyces*; SDS, sodium dodecyl sulfate; SV40, simian virus 40; *UTR*, untranslated region(s); Zf, zinc finger(s).

subunit of *Dm* Pol δ as judged by SDS-PAGE (Chiang et al., 1993). The 3'-UTR had a consensus AATAA polyadenylation signal 20 nt upstream from the polyadenylation site.

(b) The cDNA encodes *Dm* Pol δ

Three lines of evidence demonstrate that the ORF encodes *Dm* Pol δ : (i) The deduced aa sequence contains the aa sequence determined for a peptide generated from the purified 120-kDa *Dm* Pol δ , YYADPISTLDFA-SLYPSI (Fig. 1), that is strictly conserved among members of the Pol δ family (Fig. 2). (ii) A polyclonal Ab raised in chicken against *Dm* Pol δ specifically recognized a 120-kDa protein when the cDNA was expressed in *E. coli*. When the cDNA was constructed in the opposite orientation and transfected into *E. coli*, no Ab-reactive protein could be detected (data not shown). (iii) Lysates prepared from Sf9 insect cells transfected with the cDNA-baculovirus construct showed increased DNA polymerase activity as compared with lysates from the insect cells alone or insect cells transfected with wild-type baculovirus, when they were assayed with poly(dA-dT), the preferred template for Pol δ . The DNA polymerase activity in the lysates corresponded to a 120-kDa protein as judged by immunoblot analysis (data not shown).

(c) Genomic Southern and RNA transcript analysis

Genomic DNA from *Dm* adults was isolated, digested with *Hind*III, transferred to Nytran membrane, and then probed with the cDNA. A single band >12 kb was detected, indicating that there is only a single copy of the gene (data not shown).

When total RNA or mRNA isolated from *Dm* adults was probed with the cDNA, a single band of about 4 kb was detected (data not shown), indicating that only one major mRNA species corresponded to *Pol* δ .

(d) Pol δ is highly conserved

Pol δ belongs to the family of α -like DNA polymerases (reviewed in Wang, 1991). The homology among the various Pol δ species is much greater than for Pol α . Fig. 2 shows an aa sequence comparison for the catalytic subunit of all known Pol δ species. Although the N-terminal region shows the least homology, there are portions of the N-terminal region that are very conserved, e.g., NT-1 and NT-2 (Cullmann et al., 1993). Recent studies have suggested the involvement of this region in the interaction of Pol δ with PCNA. Specifically, a truncated yeast Pol δ without the N-terminal 220 aa expressed in *E. coli* did not interact with PCNA (Brown and Campbell, 1993), and studies with deletion mutants expressed in insect cells localized the region of human Pol δ that binds PCNA to the first 182 aa (Zhang et al., 1995). Furthermore, a pep-

tide containing aa 129 to 149 of human Pol δ inhibited PCNA stimulation (Zhang et al., 1995). Site-directed mutagenesis will be needed to define precisely the aa residues that mediate the interaction of Pol δ with PCNA.

The central region of the protein is the most conserved among all DNA polymerases. It contains all six α -like homology boxes (Pol boxes I to VI with box I the most and box VI the least conserved) and three Exo boxes (Fig. 2). The Pol boxes constitute parts of the polymerase active site. Studies with Pol from HSV-1 and the *Bacillus* phage ϕ 29 have demonstrated the necessity of box I for DNA polymerase activity (Dorsky and Crumpacker, 1990; Bernad et al., 1990) and substrate recognition (Marcy et al., 1990). Studies with human Pol α have also shown that box I is involved in metal-specific catalysis (Copeland and Wang, 1993). Mutations in box II/III of HSV-1 Pol and box II of human Pol α indicate that these boxes participate in dNTP (Larder et al., 1987; Tsurumi et al., 1987; Gibbs et al., 1988; Dong et al., 1993a) and primer binding (Dong et al., 1993b).

On the basis of aa sequence homology to DNA polymerase I (Pol I) of *E. coli*, it was suggested that three regions (Exo I, II and III) are related to the 3'-5' Exo activity associated with eukaryotic DNA polymerases (Bernad et al., 1989). These regions also contain conserved aa sequences that are necessary for metal, and single-stranded DNA binding (Ollis et al., 1985; Derbyshire et al., 1991). Point mutations in the predicted Exo boxes of ϕ 29 Pol resulted in a loss of 3'-5' Exo activity (Bernad et al., 1989). Recently, a new Exo I box has been proposed, and subsequently confirmed by mutational analysis of *S. cerevisiae* Pol δ (Simon et al., 1991). The original Exo I box was renamed Exo I'.

The C-terminal region of Pol δ has five conserved regions, termed CT-1, CT-2, CT-3, ZnF1 and ZnF2 (Yang et al., 1992; Cullmann et al., 1993). The function of these regions is unknown. However, the C-terminal 227 aa of the HSV-1 Pol, including CT-2 and CT-3, are necessary for interaction with the UL 42 protein (Digard and Coen, 1990), a protein that is analogous in function to PCNA (Hernandez and Lehman, 1990). No significant homology downstream from the CT-3 box could be detected between the eukaryotic Pol and the HSV-1 Pol. ZnF1 and ZnF2 are two potential zinc finger (Zf) domains that were first identified in human Pol α (Wong et al., 1988). Although the Zf regions could be good candidates for the interaction of Pol δ with PCNA (Yang et al., 1992; Cullmann et al., 1993), the N-terminal region of the protein appears to mediate this interaction as noted above. The Zf regions might therefore be involved in the interaction of the 120-kDa and 50-kDa subunits. Site-directed mutagenesis in combination with biochemical studies is clearly necessary to elucidate the function of this region.

Pf 1 MEELKTCPTNVIYGLLYDKLKEKNNDVPENVIIEFDKLLKNYERNVYDEIGNATFNKDEDLITFQID LDYTVENIFKNMIYNEGSNNILNDIYM
Mm 1 MDCKR RQGGPGVPPKRRAGHLWDEDE PPSQFQFANLALLEE IEAENRLEQEAEE LQLPEGTVGG QFSTADIDPRWRRPTLRALDPSPELIFQOLEDIDHYVGS
Bt 1 MDGKR RFGPGVPPKRRAGHLWDEDEAYRPSQFEEELALMEEM EAERRLQEQEELQSALE AADG QFSPTAIDARWLRPAPALDQPEPLIFQOLEDIDHYVAF
Hs 1 MDGKR RFGPGVPPKRRAGHLWDDDAFPPSQFEEELALMEEM EAERRLQEQEELQSVLEGVADG QVPSAIDARWLRTPTALDQPEPLIFQOLEDIDHYVAF
Dm 1 MDGKR KFNQTSNGHAKKP RNPDD EEMG FEAEALAFENSEMDQTLMLMGD PENQTT S ERWSRPPPELDPKSHNLEFQOL DVENYL
Sp 1 M TDRSNEGVLNKNY PFRNGSI HG EITDVKRRRLSERNGYGDGK SSSKEKTS FEDEL AYSQGLQ DEIKSS KDOQQWRPA
Sc 1 MSEKRS LPMVDVKKIDDEDTPQLEKKIKRQSIDHGVSPEVSTIEI IPSDSFRKYSQGFKAKTDLMTGQLESTFEQELSQMHEMDAQEEDLSSFERKKLPTDFDF

NT-1

Pf 102 PYRILLSKDKNYVPIIRIYSLRDKGCSVLINVNHPFPYF VEKPDFDINEDLIKLEMLNENLNLSQYKIEKILKIEIVKTESLMYFKKNGKDFLKITVLLP KMPVSLKXYFE
Mm 106 APPLPERPLPSRNSVPIILRAFVGTDEGFSVCCCHIQGFAPYFYPAPPFGFAGHLSELQOELNAAISRDRQGGKELSGPAVLAIELCSRESMFGYHGHPSPPLRITLALPRLMAPARR LLE
Bt 107 ARPLPAGPPSQDSVPIILRAFVGTNEGVSVCCHIHGFAPYFYPAPPFGFAGHLSELQOELNAAISRDRQGGKELSGPAVLAELCSRESMFGYHGHPSPPLRITLALPRLMAPARR LLE
Hs 108 AQPVPGGPPPSRGSVPIILRAFVGTDEGFSVCCCHIHGFAPYFYPAPPFGFAGHMDLQRELNLAISRDRSGRRELTPAVLAVELCSRESMFGYHGHPSPPLRITLALPRLVAPARR LLE
Dm 88 GQPLPGMPGAQIGHVPPVRFMGVTHMEGNSVCCVHGFQCPYPIEAPSQFEEHCEKQLKALDQKVIADIRNNKNDVQEAFLMVELVEKLNINHGNGDKKQRYIKISVTLPRFVAASR LLK
Sp 88 LPAINPEKDDIYPCIDSEEFTEGVSPIRLFGVTDNGNSILVHVGFLLPYFVKAAPVGFPEMERLERTQDLDATCNGGVIDHICIEMKENLYGFQGNKSPFIKIFTTNPRILSRANVFE
Sc 109 SLYDISFQOIDADCSVLNIGIKDENSTVVRFFGVTSSEHSLVCLNVTGFKNYLVPAPNSSDANDQEQINKFVHYLNETFDHAIDSIIEVVSQIGSITWQSDTKLPFWKIYVYPHMVNKLRTA

NT-2

Pf 221 GIVHV NNSKIGGIVYEANLPIIRYIIDHKITGSSWINCKKHYIERNKKNKISNCTFEIDISYEHVPEITLENEYQOIPYRILSFDIECIKLDKGGKFEAKNDPIIQISILYFQG
Mm 227 QGVRVPLGLTSPFAFYEANVDFEIRFMVDADIVGCNWLLELP AGKYVRAEAKKATLQCEADVLDVSDVISHPPEQWQRIAEFLRVSFDIEC AGRKGIFPEPERDPVYIQICSLGLRWG
Bt 228 QGIRLAGLGTSPFAFYEANVDFEIRFMVDADIVGCNWLLELP AGKYVLRPEKATLQCEADVLDVSDVISHPPEQWQRIAEFLRVSFDIEC AGRKGIFPEPERDPVYIQICSLGLRWG
Hs 229 QGIRVAGLGTSPFAFYEANVDFEIRFMVDADIVGCNWLLELP AGKYVLRKKEKATLQCEADVLDVSDVISHPPEQWQRIAEFLRVSFDIEC AGRKGIFPEPERDPVYIQICSLGLRWG
Dm 209 KEVINSEIDPQDCRAFENNIDPDIRFMVDADIVGCNWLLELPMGHWRIRKSHSKPLPESRQIEAVDVAFDRIISHPPEGQWKAIFRILSFDIEC AGRKGIFPEAKIDPVIQIANMVIROG
Sp 210 RGEFNFEEFLFPVGVGVTTFESNTQVLLRFMIDCDVVMNWIHLPAKSY QFRYQNRVSNQIEIWDVNIYKDLISLPAEQQWKMALRIMSFDIEC AGRKGIFPEPDSIDPVIQIASIVTQYG
Sc 231 FERGHLSFNSWFSNCTTT YDNIAYTLRLMVDCCGIVGMSWITLPGKYSIPIENNRVSSQCLEVSYNRYNLAHPAEGDWSHTAELRIMSFDIEC AGRIGVFEPEYDPVIQIANVVSIAG

Exo I

Exo I Pol IV Exo II

Pf 339 EPIDNCTKFIPTLEECASIPGNSVIFWNEKTLLEAWNEFIIRIDDFLITGYNIINFDLPYILNRFIHALNKLKFLGRINKVASTVKDSSFSKQFQTHETKEINIFGRIQFDVYDLIKRD
Mm 344 EP EFLRLALTLRCPAPILGAKVQSYEREDELLQAWADFILAMDQVITGYNIQNFDPYILISFACALQKVDPRFPFLGRVTLGRSNI RDSFQSRQVGRDRSKVISMVGRVQMDMLQVLLRE
Bt 345 EP EFLRLALTLRCPAPILGAKVQSYEREDELLQAWSTFIRIMDDQVITGYNIQNFDPYILISFACALQKVPGLGRVIGLRSNI RDSFQSRQVGRDRSKVISMVGRVQMDMLQVLLRE
Hs 346 EP EFLRLALTLRCPAPILGAKVQSYEREDELLQAWSTFIRIMDDQVITGYNIQNFDPYILISFACALQKVPGLGRVIGLRSNI RDSFQSRQVGRDRSKVISMVGRVQMDMLQVLLRE
Dm 330 E REPFIRNVTIATLDFNSLYPSIMAHNLCYTLLRPGAAQKL GLKDFEIKTPTGDFEVKSSVRKGLLPOILENLSARKRAKALAKETDPLRRQVLDGRQLALKVANSVYGF
Sp 330 DSTPFVRNVCVDCTSQIVGQVYEFQNAEMLSWSKFRVDRDQVILGYNICNFDIYLLDRAKSLRIHNPLLRGIHNFVSQAKETSFSKAYGTRESKTTISIPGRQLDMLQVMDR
Sc 351 AKKFFIRNVTIATLDFNSLYPSIMAHNLCYTLLRPGAAQKL GLKDFEIKTPTGDFEVKSSVRKGLLPOILENLSARKRAKALAKETDPLRRQVLDGRQLALKVANSVYGF

Exo III

Pf 461 YKLSYTLNYSVFEFLKEQKEDVHYSIMNDLQNESPESRKRRIATYCIKDGVLPLRILDKLLFIYNYEMARVGTGPFYLLTRGQQIKVTSQLYRKCKELNYPSTYMKVNTNEKYGATV
Mm 465 HKLRSYTLNAVSHFLGEQKEDVHYSITDLQNGDQTRRLAVYCLKDAYLPLRILERLMLVNNVEMARVGTGPLYLLTRGQQIKVTSQLYRKCKELNYPSTYMKVNTNEKYGATV
Bt 466 YKLSYTLNAVSHFLGEQKEDVHYSITDLQNGDQTRRLAVYCLKDAYLPLRILERLMLVNNVEMARVGTGPLYLLTRGQQIKVTSQLYRKCKELNYPSTYMKVNTNEKYGATV
Hs 467 YKLSYTLNAVSHFLGEQKEDVHYSITDLQNGDQTRRLAVYCLKDAYLPLRILERLMLVNNVEMARVGTGPLYLLTRGQQIKVTSQLYRKCKELNYPSTYMKVNTNEKYGATV
Dm 451 YKLSYTLNAVSHFLGEQKEDVHYSITDLQNGDQTRRLAVYCLKDAYLPLRILERLMLVNNVEMARVGTGPLYLLTRGQQIKVTSQLYRKCKELNYPSTYMKVNTNEKYGATV
Sp 451 YKLSYTLNAVSHFLGEQKEDVHYSITDLQNGDQTRRLAVYCLKDAYLPLRILERLMLVNNVEMARVGTGPLYLLTRGQQIKVTSQLYRKCKELNYPSTYMKVNTNEKYGATV
Sc 472 YKLSYTLNAVSHFLGEQKEDVHYSITDLQNGDQTRRLAVYCLKDAYLPLRILERLMLVNNVEMARVGTGPLYLLTRGQQIKVTSQLYRKCKELNYPSTYMKVNTNEKYGATV

Pol II

Pol VI

Pol III

Pf 583 IEPKRGYVDPVIAITLDFNSLYPSIMAHNLCYTLLRPGAAQKL GLKDFEIKTPTGDFEVKSSVRKGLLPOILENLSARKRAKALAKETDPLRRQVLDGRQLALKVANSVYGF
Mm 585 IEPKRGYVDPVIAITLDFNSLYPSIMAHNLCYTLLRPGAAQKL GLKDFEIKTPTGDFEVKSSVRKGLLPOILENLSARKRAKALAKETDPLRRQVLDGRQLALKVANSVYGF
Bt 586 IEPKRGYVDPVIAITLDFNSLYPSIMAHNLCYTLLRPGAAQKL GLTDDQFKTPTGDFEVKSSVRKGLLPOILENLSARKRAKALAKETDPLRRQVLDGRQLALKVANSVYGF
Hs 587 IEPKRGYVDPVIAITLDFNSLYPSIMAHNLCYTLLRPGAAQKL GLTDDQFKTPTGDFEVKSSVRKGLLPOILENLSARKRAKALAKETDPLRRQVLDGRQLALKVANSVYGF
Dm 572 IEPKRGYVDPVIAITLDFNSLYPSIMAHNLCYTLLRPGAAQKL GLTDDQFKTPTGDFEVKSSVRKGLLPOILENLSARKRAKALAKETDPLRRQVLDGRQLALKVANSVYGF
Sp 572 IEPKRGYVDPVIAITLDFNSLYPSIMAHNLCYTLLRPGAAQKL GLTDDQFKTPTGDFEVKSSVRKGLLPOILENLSARKRAKALAKETDPLRRQVLDGRQLALKVANSVYGF
Sc 593 IEPKRGYVDPVIAITLDFNSLYPSIMAHNLCYTLLRPGAAQKL CNKATVERLNLKIDEDYVITPNGDYVPTTKRRFGLPIILDELISARKRAKALDRDEKDFPKRDLVLDGRQLALKVANSVYGF

Pol I

Pf 702 GASSGQLPCELEAVASITTLGEMIEKTKERVESFYCKSNYEHNSVIYGDGDSVMKFGVTNNIEEAMTLGKDAERISKEFLSPIKLEFEKVCYPLLNNKKRYAGLL YTNPNKHFKMD
Mm 703 GA QVGLRPLCELEISQSVTGGFQEMIEKTKQLVESKYTVENGYSASAVVYGDGDSVMCRFGVSSVAEAMALGREAADVWSGHFSPPIRLEFEKVVFPYLLISKKRYAGLLFSRSDAHDKMD
Bt 704 GA QVGLRPLCELEISQSVTGGFQEMIEKTKQLVESKYTVENGYSASAVVYGDGDSVMCRFGVSSVAEAMALGREAADVWSGHFSPPIRLEFEKVVFPYLLISKKRYAGLLFSRSDAHDKMD
Hs 705 GA QVGLRPLCELEISQSVTGGFQEMIEKTKQLVESKYTVENGYSASAVVYGDGDSVMCRFGVSSVAEAMALGREAADVWSGHFSPPIRLEFEKVVFPYLLISKKRYAGLLFSRSDAHDKMD
Dm 694 GA QVGLRPLCELEISQSVTGGFQEMIEKTKQLVESKYTVENGYSASAVVYGDGDSVMYNGVKTLEMSBLEGREAALVWSKVFHPHPIKLEFEKVVFPYLLISKKRYAGLL YTRPDTYTKMD
Sp 691 GA TNGRLPCLAISSSVTSYGRQEMIEKTKQVVEKRYRIENGYSDAVVIYGDGDSVMKFGVKTLPKAMKLEEAANVSDQFPNPI WSPSTFPYLLISKKRYAGLL FWTTRDTYTKMD
Sc 712 GA TVGKLPCLAISSSVTAYGRQEMILKTKTAVQEKYICRNGYKHDAVVIYGDGDSVMKFGVTDLKEAMDLTGTEAAKYVSTLFPKHPIINLEFEKAYFPYLLINKRYAGLL FWTNPDKFKLD

Pol V

CT-1

CT-2

Pf 823 CKGLETVRRDNCPLVANLVTSASLRLLIDRDPEGAHAQDVIISDLLCNRIDISQLVITKELTRAAADYAGKQHVLEAERMRKRDPGSAPSLGDRVPYVISAARGVAAYMKSIEDPLFVLE
Mm 824 CKGLEAVRRDNCPLVANLVTSASLRLLIDRDPEGAHAQDVIISDLLCNRIDISQLVITKELTRAAADYAGKQHVLEAERMRKRDPGSAPSLGDRVPYVISAARGVAAYMKSIEDPLFVLE
Bt 825 CKGLEAVRRDNCPLVANLVTSASLRLLIDRDPEGAHAQDVIISDLLCNRIDISQLVITKELTRAAADYAGKQHVLEAERMRKRDPGSAPSLGDRVPYVISAARGVAAYMKSIEDPLFVLE
Hs 826 CKGLEAVRRDNCPLVANLVTSASLRLLIDRDPEGAHAQDVIISDLLCNRIDISQLVITKELTRAAADYAGKQHVLEAERMRKRDPGSAPSLGDRVPYVISAARGVAAYMKSIEDPLFVLE
Dm 814 CKGLETVRRDNCPLVANLVTSASLRLLIDRDPEGAHAQDVIISDLLCNRIDISQLVITKELTRAAADYAGKQHVLEAERMRKRDPGSAPSLGDRVPYVISAARGVAAYMKSIEDPLFVLE
Sp 809 SKGLETVRRDNCPLVSYVIDTALRMLIDQDVEGAQLFTKVIISDLLQNKIDMSQHVITKAL SKTDYAAKMHVLEAERMRKRDGASAPAGDRVAVYVISAARGVAAYMKSIEDPLFVLE
Sc 832 QKGLASVRRDSCSLVSVMMNKVLKLLIERNVGGA LAFVRETINDILHNRVDISKLIISKTLAPNYTNPQHVLAERM KRREGVGNVDRDQVYVISAARGVAAYMKSIEDPLFVLE

CT-3

Pf 943 NNLAIYDNYHLDAIKSPLSRIFE VIMQNSDSLFGDHTRHKTILTSSQTALSKFLKKSVCRCIGC NSSIKKPP LCNHCKENKHFSIYMQKIKDFKNQNEFFQLWTEBCRCQGNL
Mm 946 HSLPIDTQYYLEQQLAKPLLRIFEPILGEGRAEAVLLRGDHTCRKTVLTKGVGGLAFAPKRRNCICIGC RSVIDHQGAVCKFCQPRESELYQKEVSHLN ALEERFSLRWTCQRCQGS
Bt 947 HSLPIDTQYYLEQQLAKPLLRIFEPILGEGRAEAVLLRGDHTCRKTVLTKGVGGLAFAPKRRNCICIGC RTVLSHQGAVCKFCQPRESELYQKEVSHLN ALEERFSLRWTCQRCQGS
Hs 948 HSLPIDTQYYLEQQLAKPLLRIFEPILGEGRAEAVLLRGDHTCRKTVLTKGVGGLAFAPKRRNCICIGC RTVLSHQGAVCEFCQPRESELYQKEVSHLN ALEERFSLRWTCQRCQGS
Dm 934 NSVPIDATYYLEQQLSKPLLRIFEPILG DNAESILLKGHTRTTRVTVTKVGLGAGFMKTKTCLGCKSLMPKQYEQACLCPHCEPRMSLEYQKEVGAQR ELEETFSRLWTECQRCQESL
Sp 929 NNIPIDAKYYLENQLSKPLLRIFEPILG EKASSLLHGHTRTTISMAAPSVGIMKFAVKVETCLGCKAFIKGG KTALECNLNRSAEYLRQVAVQN DLEVRFARLWTCQRCQGS
Sc 948 NNIQVDSRYLLTQNLQNPISIVAP IGDKQANGMVFVKSIRKINTGSGQKGLMSFIIKVEACKSCKGFLRGG EGPLCSNCLARSSELYIKALYDVR DLEEKYSLRWTCQRCQGNL

Pf 1057 HVDVICMNRDCPIFYRRAKIKKD IANLQEQVTSLRMDW
Mm 1064 HEDVICTSRDCPIFYMRKVRKDELDQERLLRQFGPPGPEAW
Bt 1065 HEDVICTSRDCPIFYMRKVRKDELDQERLLRFGPPGPEAW
Hs 1066 HEDVICTSRDCPIFYMRKVRKDELDQEQQLRFGPPGPEAW
Dm 1054 HEVICSNRDCPIFYMRQKVRMDLDNQEKRRVLRFLAEW
Sp 1064 HQDVICTSRDCPIFYMRAEHKLLQSQVDLLKRFDEMSW
Sc 1066 HSEVICSNRDCPIFYMRKVRKDELDQEQVQLSKW

Fig. 2. Amino-acid sequence comparison of several Polδ. The homology boxes and conserved Cys residues of putative ZI are indicated. Sources for the sequences were Drosophila melanogaster (Dm, this work), Homo sapiens (Hs, Chung et al., 1991), Bos taurus (Bt, Zhang et al., 1991), Mus musculus (Mm, Cullmann et al., 1993), Saccharomyces cerevisiae (Sc, Boulet et al., 1989), Schizosaccharomyces pombe (Sp, Pignède et al., 1991) and Plasmodium falciparum (Pf, Ridley et al., 1991).

(e) Conclusions

(1) A full-length cDNA encoding *Dm* Pol δ has been isolated using a combination of PCR and cDNA library screening.

(2) The deduced ORF contains all the homology boxes for Pol δ .

(3) A comparison of the deduced aa sequence with those from other sources indicate that Pol δ is very highly conserved.

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