Aphidicolin inhibits DNA polymerase II of *Escherichia coli*, an alpha-like DNA polymerase

H.Chen[1], C.B.Lawrence[1], S.K.Bryan and R.E.Moses[1,*]
Department of Cell Biology and [1]Institute for Molecular Genetics, Baylor College of Medicine, Houston, TX 77030, USA

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The *polB* gene of *E. coli* encodes DNA polymerase II. We have reported the cloning (1, 2) and sequencing of the *polB* gene (3) (GenBank Accession number M35371). DNA polymerase II appears to be a non-essential function since the *polB* gene is missing from some widely used strains (3). Several sub-families of DNA polymerases have been identified (4). In this communication, we demonstrate that DNA polymerase II of *E. coli* is an α-like DNA polymerase by amino acid sequence conservation and inhibition by aphidicolin.

A search of the MBCRR Protein Pattern Library indicated significant similarity (6.3 standard deviations above the mean of comparisons with a negative control set) to only a single pattern (pattern 156) generated from a set of α-like polymerases (Fig. 1). DNA polymerase II has the highest overall similarity to human DNA polymerase α and phage T4 DNA polymerase. The entire length of DNA polymerase II is similar to a subregion of both polymerases and within this region the sequences are both approximately 22% identical. There are three sequence motifs characteristic of the α-like family (regions I, II, III) (4, 5). The H region shown in Figure 1 corresponds to region I characterized by the presence of 5−7 contiguous conserved amino acids (YGDTDS). The E region corresponds to region II. In addition to a central conserved sequence of 5−10 residues (DSLYPS), there are additional sequence identities on both sides of the conserved center. Regions F and G correspond to region III with invariant residues at five positions. These three regions are in the same linear arrangement (II-III-I) as other DNA polymerases in this family and the relative distances between the regions are similar.

The N-terminal region (residues 1−301) of DNA polymerase II has significant similarity to only a subset of the α-like DNA polymerases (human α, phage T4, yeast polIII, Epstein-Barr virus, and *Autographa californica* nuclear polyhedrosis virus [A-CNPV]). The regions of the six polymerases having the highest mutual similarity were aligned (shaded boxes in Figure 1). Region A in other α-like polymerases has 3′−5′ exonuclease activity.

Human DNA polymerase α is inhibited by the drug aphidicolin. We found that aphidicolin also inhibited DNA polymerase II (Ki = 50 μM). The peculiar aphidicolin sensitivity of DNA polymerase II offered an opportunity to assess its possible role in DNA replication. Toluene-treated *E. coli* cells offer a convenient means of assessing the effect of compounds on the elongation phase of DNA replication, since the permeable cells eliminate problems of transport (6). We found no significant effect of aphidicolin on the level of ATP-dependent synthesis in seven different strains of *E. coli*, including two strains apparently lacking the *polB* gene (3), MC1061 and MC1000. We conclude that there is no detectable role of DNA polymerase II in the elongation phase of DNA replication.

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* To whom correspondence should be addressed

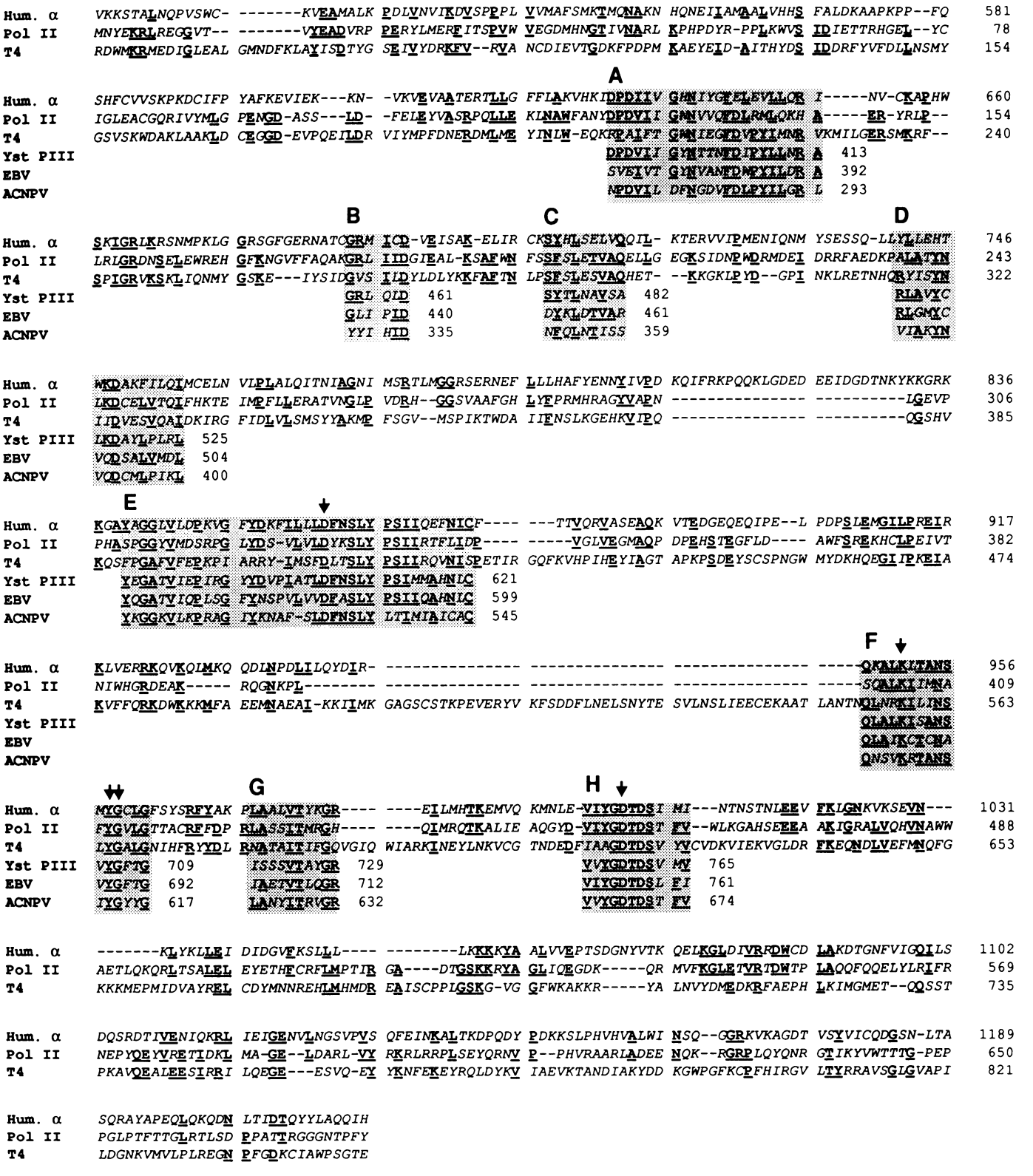

Figure 1. Composite of several pairwise and multiple alignments between α-like DNA polymerases. This figure shows the alignments of selected regions of the following polymerases (reference or SWISS-PROT ID, or GenPept Locus): *E. coli* DNA polymerase II, human alpha (DPOA$HUMAN), T4 (DPOL$BPT4), yeast polIII large subunit (DPO3$YEAST), Epstein-Barr virus (DPOL$EBV), *Autographa californica* nuclear polyhedrosis virus (Gen-Pept Locus: NPADNAPMA 1). Human alpha and T4 polymerases were aligned pairwise with DNA polymerase II and the composite of the two alignments is summarized in the figure. Selected regions of all six polymerases were aligned using the multiple alignment method of Smith and Smith (7). These regions are shaded in the figure. Amino acids present in one-half or more of the sequences in any region are underlined. Positions proposed to be invariant in all polymerases (5) are indicated with an arrow. Position coordinates are the number of amino acids from the N-terminal amino acid as given in the indicated data base entry.