DNA polymerase delta is involved in many aspects of DNA metabolism, including various types of repair, and both leading and lagging strand elongation. In Saccharomyces cerevisiae, the DNA polymerase delta complex consists of three subunits of 125, 55, and 40 kDa. An additional small fourth subunit is found in the enzymes from fission yeast and human. Hydrodynamic studies of S. cerevisiae DNA polymerase delta have revealed the three-subunit complex to be highly elongated, with an unusual sedimentation constant. This deviation from globularity of the entire three-subunit complex could be attributed to the extremely elongated structure of the third subunit, Pol32p. Indeed, Pol32p with a calculated molecular mass of 40 kDa migrates as a 55-kDa protein by SDS-PAGE, while 55-kDa Pol31p migrates as a 58-kDa protein. The catalytic subunit and the second subunit of DNA polymerase delta, Pol3p and Pol31p, are highly conserved in eukaryotes. These genes are also essential in both budding and fission yeast. In contrast, the third subunit, Pol32p, is not essential, and shows an extreme divergence at the primary amino acid level, such that in Saccharomyces paradoxus, which shows a mean amino acid sequence identity with S. cerevisiae of over 90% for all proteins, only 82% sequence identity is observed for Pol32p. For the Pol3p and Pol31p subunits, sequence identity is 96% and 93%, respectively. The essential functionalities of Pol32p reside in a small amino-terminal domain, not much larger than that necessary to specify its interaction with Pol31p. Compared to fission yeast and human, only one motif is highly conserved in Pol32p, the consensus PCNA-binding motif QXXXXFF at the extreme carboxy-terminus. pol32delta mutants show severe defects in DNA replication, repair, and mutagenesis. Further, the combination of pol32delta with conditional mutations in POL3, POL31, or POL30is lethal.