The Ku heterodimer is conserved in a wide range of eukaryotes and plays multiple roles in DNA metabolism. Composed in yeast of Yku70p and Yku80p, Ku functions in genome stability by participating in pathways for DNA double-strand breakrepair via nonhomologous end-joiningand telomere maintenance. Ku is also involved in nuclear spatial organization and in the formation of gross chromosomal rearrangementsincluding translocations, deletions, inversions, amplifications, and aneuploidy. Ku is a multifunctional protein that has distinct activities at DSBs and telomeres, including roles in the recruitment of telomerase and telomere length homeostasis, protection of telomeric ends from nucleolytic degradation and homologous recombination, formation of telomeric heterochromatin leading to transcriptional silencing of nearby genes, late firing of replication origins near telomeres, and nuclear localization of telomeres. In all eukaryotes, the Ku heterodimer is encoded by duplicate copies of an ancestral gene. The defining feature of Ku, observed in crystals of the human protein and inferred by conservation in yeast, is its central beta-barrel ring structure. Ku binds DNA by slipping the DSB end through this ring, which accounts for the substrate specificity of Ku as well as its ability to translocate along the DNA duplex. The Ku ring appears to bind ends in only one orientation, indicating an inherent polarity in its other domains. The C terminus of Yku80p is positioned toward the DSB end and makes a contact with Dnl4p that is important for NHEJ. The C terminus of Yku70p is oriented away from the DSB end. Correspondingly, this 25 amino acid region of Yku70p is required not for NHEJ but for telomere functions. In general, the yeast Ku C termini are more rudimentary than in higher eukaryotes, in that Yku80p lacks an alpha-helical bundle, whereas Yku70p lacks an SAP domain.Inactivation of YKU70 or YKU80 results in telomere shortening, loss of telomere clustering and silencing, deregulation of the normally cell cycle-dependent telomeric G overhang, earlier activation of replication origins close to telomeres, and synthetic lethality with mutations that impair telomere replication. Certain yku80 C-terminal mutations have been shown to impair NHEJ while telomeric functions are retained. Conversely, yku80 alleles have been identified that are proficient in NHEJ but defective in specific aspects of telomeric function. In humans, inactivation of either subunit of the Ku70-Ku80 heterodimer generates severe defects such as sensitivity to DNA damage, telomere shortening, and increased GCRs that are frequently observed in many cancers.