Pnkp kinase family, and includes both dna and

PNKP is essential for maintaining genomicstability. To date, PNKP is the only DNA repair enzyme that has been identifiedas possessing 5?-kinase activity (59). In addition, the 3?-phosphatase activity ofPNKP supersedes that of APE1 and aprataxin (APTX), which have been identifiedas being able to dephosphorylate 3?-phosphate (59). Previous studies revealed major differences in substrate preferencebetween the kinase and phosphatase domains, and it also showed that bothfunction independently of one another, with the phosphatase having a fastercatalytic rate (103). The kinase domain, which is selective forDNA, preferentially phosphorylates nicks, small gaps and recessed 5?-hydroxylends with a 3?-overhang (3, 98, 104).

Furthermore, the kinase domain can bind todouble-stranded 5?-termini without base pair disruption (105). This domain belongs to the adenylate kinase family, and includesboth DNA and ATP binding sites. The ATP binding site consists of Walker A(P-loop) and B motifs (98, 106, 107). The Walker A motif binds the ?- and?-phosphates of ATP, while the Walker B motif coordinates Mg2+ ion. The DNA end is sequestered in a pocket of the protein with access to the 5?-OHterminus and the reaction is catalyzed by the Asp397 residue (Figure 1. 5A) (108). The phosphatasedomain belongs to the haloacid dehalogenase (HAD) superfamily andcontains a conserved DxDGT motif (93, 109).

This domain is dependent on Mg2+, whichstabilizes the negative charge on the substrate phosphate during catalysis, andcatalyzes the removal of 3?-phosphate of DNA (110). Thephosphatase domain utilizes a two-step mechanism, the substrate phosphate isfirst held in place for nucleophilic attack by the Asp171 carboxylate togenerate the covalent phospho-

aspartate intermediate. In the second step, thephospho-aspartate is hydrolyzed by Asp172, which deprotonates the attackingwater molecule (93, 110). The dephosphorylation process acts in thesame manner on nicked and gappeddouble-stranded substrates and single-stranded substrates as small as 3nucleotides (86, 93, 105, 108, 110). However, the binding of double-stranded DNA to the PNKP phosphatase domain destabilizes base pairing in the two or threeterminal base pairs of double-stranded substrates closest to the 3?-phosphateby electrostatic interactions between a positively charged surface of PNKP andthe DNA strand (105). PNKP kinase andphosphatase activities are active on DNA and inactive on RNA. Mammalian cells have distinct DNA-specificand RNA-specific polynucleotide kinase activities. The mammalian DNA kinase hasa pH optimum of 5.

5, while the RNA kinase has an alkaline pH optimum (111-114). Moreover, the human RNA kinase does not havean associated 3?-phosphatase, whereas DNA kinases do have an inherentDNA-specific 3?-phosphatase function (115, 116).