Nudt16, previously called X29, is known to be a metal-dependent decapping enzyme expressed in mammals and *Xenopus* . Nudt16 has been proposed to be the catalytic component of a previously unknown nuclear RNA degradation pathway that degrades nuclear-limited RNAs. However, nothing was known about the function of the protein in other species. This work proposes to examine whether the sequence and, more importantly, the RNA decapping function of the Nudt16 protein is conserved across evolution. The hypothesis tested here is that if the protein and decapping function are conserved then the proposed *in vivo* role of a nuclear pathway for RNA degradation is more likely to be true.

The experiments demonstrate that the sequence of the Nudt16 protein is evolutionarily conserved across metazoans. Additionally, there is evidence for a gene duplication that occurred at the level of tetrapods resulting in a cytoplasmic protein lacking RNA decapping function. Functional analysis of putative orthologs of Nudt16 protein from several divergent organisms demonstrated that the ability to act as an RNA decapping protein is well conserved.

More complete characterization of the Nudt16 protein from *Xenopus* as well we several of the other organisms demonstrated that the protein functions as homodimer with up to two RNA binding sites that may act independently of one another. While metal is a required co-factor, characterization of additional orthologs demonstrated that there is broad RNA substrate specificity for RNA decapping in the presence of both Mg^{+2}

and Mn^{+2}. Identification of putative *in vivo* interacting partners of Nudt16 was performed using co-immunoprecipitations. Three proteins were identified but still need to be verified. Collectively, these data demonstrate that Nudt16 is conserved both in sequence and *in vitro* function across evolution and support the existence of a nuclear RNA degradation pathway for nuclear-limited RNAs in Metazoans.