Nudt16, previously called X29, is a metal-dependent decapping enzyme. In frogs and mammals, Nudt16 has been proposed to be the catalytic component of a previously unknown nuclear RNA degradation pathway degrading nuclear-limited RNAs. This work examines whether the sequence and RNA decapping function of the Nudt16 protein is conserved across evolution. The hypothesis is that if the protein and decapping function are conserved then the proposed in vivo nuclear RNA degradation pathway is more likely to be true.

The experiments demonstrate that the sequence of the Nudt16 protein is evolutionarily conserved. There is evidence for a gene duplication that resulted in a cytoplasmic protein lacking RNA decapping function. Functional analysis of putative orthologs of Nudt16 protein from diverse organisms demonstrated that RNA decapping activity is conserved.

More complete characterization of the Nudt16 protein from frogs as well as several other organisms demonstrated that the protein functions as a homodimer with two RNA binding sites that act independently of one another. While metal is a required co-factor, characterization of additional orthologs demonstrated that in the presence of either Mg$^{2+}$ or Mn$^{2+}$ multiple RNAs can be substrates. 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, supporting the existence of a nuclear RNA degradation pathway for nuclear-limited RNAs.