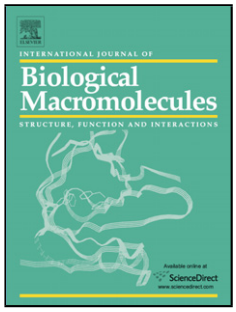

Title: Cooperativity and evolution of *Tetrahymena* two-domain arginine kinase

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Tetrahymena pyriformis contains two arginine kinases, a 40-kDa enzyme (AK1) with a myristoylation signal sequence at the N-terminus and a two-domain 80-kDa enzyme (AK2). The former is localized mainly in cilia and the latter is in the cytoplasm. AK1 was successfully synthesized using an insect cell-free protein synthesis system and subjected to peptide mass fingerprinting (PMF) analysis. The masses corresponding to unmodified N-terminal tryptic peptide or N-terminal myristoylated peptide were not observed, suggesting that N-terminal peptides were not ionized in this analysis. We performed PMF analyses for two other phosphagen kinases (PKs) with myristoylation signals, an AK from *Nematostella vectensis* and a PK from *Ectocarpus siliculosus*. In both cases, the myristoylated, N-terminal peptides were clearly identified. The differences between the experimental and theoretical masses were within 0.0165–0.0583 Da, supporting the accuracy of the identification.

Domains 1 and 2 of *Tetrahymena* two-domain AK2 were expressed separately in *Escherichia coli* and the extent of cooperativity was estimated on the basis of their kinetic constants. The results suggested that each of the domains functions independently, namely no cooperativity is displayed between the two domains. This is in sharp contrast to the two-domain AK from *Anthopleura*.