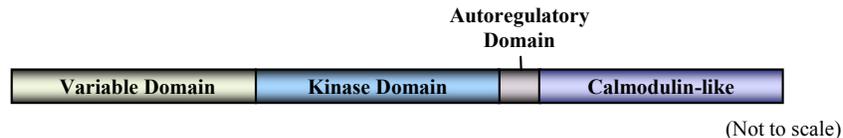


## Calcium-dependent protein kinases and related proteins in *Physcomitrella patens*

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Calcium-dependent protein kinases (CDPKs) are serine/threonine kinases that are found in all plants and in the protist subgroup Apicomplexa. CDPKs are composed of four domains: the variable domain, the catalytic domain, the autoregulatory domain, and the calmodulin-like domain. The variable domain differs greatly in length and amino acid composition between individual CDPKs. This domain also often contains sites for the addition of the fatty acids myristate and palmitate, which function to target the CDPK to cellular membranes. The catalytic domain contains all of the conserved amino acids of a typical serine/threonine kinase. The autoregulatory domain inhibits activity of the catalytic domain by acting as a pseudosubstrate. This inhibition is released when calcium binds the calmodulin-like domain, which contains four calcium-binding EF-hands. Some CDPKs are induced by stresses such as salt, drought and pathogen attack, thus implicating CDPKs in signaling pathways in response to environmental stimuli; however, the functions of the majority of CDPKs are still unknown.



Seventeen CDPKs have been identified and sequenced in *Physcomitrella patens*. Phylogenetic analysis shows that CDPKs form three subgroups, though the significance of these groups is still unknown. Analysis of the variable domains reveals that fourteen of the CDPKs possess a potential N-terminal myristoylation site. Nine of these proteins also contain either one or two predicted palmitoylation sites closely following the myristoylation site. A similar arrangement of acylation sites has been observed in many CDPKs in vascular plants.

Two members of the CDPK family (*CPK4* and *CPK6*) were expressed in *E. coli* and an *in vitro* kinase assay was performed. Enzyme activity was stimulated in the presence of calcium, confirming that these kinases are calcium-dependent. The expression pattern of *CPK6* was analyzed using stable transformants containing a GUS reporter gene fused to the *CPK6* upstream regulatory region. Preliminary results show strongest expression in axillary hair cells and in young phyllids. Weaker expression was seen in mature phyllids and in protonema.

In vascular plants, there are two classes of genes that are closely related to CDPKs: the CDPK-related kinases (CRKs) and the calcium and calmodulin dependent protein kinases (CCaMKs). Representatives of both of these families have also been identified in *P. patens* suggesting that all three types of kinases arose before the evolution of vascular plants.