

## Cellular localization and biochemical characterization of a novel calcium-dependent protein kinase from tobacco

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### ABSTRACT

By screening tobacco cDNA library with *MCK1* as a probe, we isolated a cDNA clone *NtCPK5* (accession number AY971376), which encodes a typical calcium-dependent protein kinase. Sequence analyses indicated that *NtCPK5* is related to both CPKs and CRKs superfamilies and has all of the three conserved domains of CPKs. The biochemical activity of *NtCPK5* was calcium-dependent. *NtCPK5* had  $V_{max}$  and  $K_m$  of 526 nmol/min/mg and 210  $\mu$ M respectively with calf thymus histone (fraction III, abbreviated to histone IIIs) as substrate. For substrate syntide-2, *NtCPK5* showed a higher  $V_{max}$  of 2008 nmol/min/mg and a lower  $K_m$  of 30  $\mu$ M. The  $K_{0.5}$  of calcium activation was 0.04  $\mu$ M or 0.06  $\mu$ M for histone IIIs or syntide-2 respectively. The putative myristoylation and palmitoylation consensus sequence of *NtCPK5* suggests that it could be a membrane-anchoring protein. Indeed, our transient expression experiments with wild type and mutant forms of *NtCPK5*/GFP fusion proteins showed that *NtCPK5* was localized to the plasma membrane of onion epidermal cells and that the localization required the N-terminal acylation sites of *NtCPK5*/GFP. Taking together, our data have demonstrated the biochemical characteristics of a novel protein *NtCPK5* and its subcellular localization as a membrane-anchoring protein.

**Keywords:** calcium-dependent protein kinase, phosphorylation, GFP, subcellular localization.

### INTRODUCTION

Calcium ( $Ca^{2+}$ ) as a ubiquitous signal molecule plays vital roles in plant response to various stimuli, including light, environmental stresses, pathogen attack, and hormones by fluctuation of cytosolic  $Ca^{2+}$  concentration in all living cells [1]. In plants, different calcium signal was transduced through several calcium-binding proteins such as calmodulin (CaM), calcium-dependent protein kinase (CDPK or CPK), calcineurin B-like protein (CBL) and chimeric  $Ca^{2+}$ /CaM-dependent protein kinase (CCaMK) into downstream responses, including altered protein phos-

phorylation and gene expression patterns [2-4]. CPKs are a group of well-characterized protein kinases and have been found in many plants including Arabidopsis, soybean, rice, tobacco and potato [5]. CPKs contain three conserved domains: kinase catalytic domain, junction domain and calmodulin-like domain. Junction domain can serve as a pseudosubstrate and block the catalytic pocket of kinase domain in the absence of calcium. Upon the calcium binding to EF-hands, junction domain (the autoinhibitory region) is released from the kinase domain to activate CPKs [6].

Except of the three highly conserved domains, CPKs also have an N-terminal variable region, which often contains N-terminal acylation (including myristoylation and palmitoylation) sites required for the subcellular localization of certain CPKs [7-10]. Myristoylation often occurs at Gly residue at position 2 and may or may not sufficient for membrane anchoring. Palmitoylation occurred at Cys residue at position 4 as a supplement can promote and stabilize the targeting of CPKs to plasma membrane [7-9]. In Arabidopsis, there are 34 CPK isoforms, among them, only five AtCPKs lack both Gly2 and Cys4. Different N-terminal acylation from variable N-terminal sequences produces isoform-specific subcellular localization of AtCPKs, sug-

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**Abbreviations:** CPK or CDPK (Ca<sup>2+</sup>-dependent protein kinase); CRK (CPK-related kinase); CBK (calmodulin-binding protein kinase); CaMK (Ca<sup>2+</sup>/CaM-dependent protein kinase); CCaMK (chimeric Ca<sup>2+</sup>/CaM-dependent protein kinase); MCK (maize homologue of mammalian CaMK); CBL (calcineurin B-like protein); GFP (green fluorescent protein); histone IIIs (calf thymus histones fraction III); Ni-NTA (Ni<sup>2+</sup>-nitrilotriacetate).

# Biochemical Approaches To Cellular Calcium

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