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Identification and Characterization of Nuclear Localization Signals of CaMKP-N

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Calmodulin-dependent protein kinase phosphatase (CaMKP) and CaMKP-N dephosphorylate and regulate multifunctional Ca^{2+}/calmodulin-dependent protein kinases. The enzymatic properties of CaMKP-N and CaMKP resemble each other, whereas their localizations are different. CaMKP-N is localized in the nucleus, whereas CaMKP is localized in the cytosol. In the present study, the nuclear localization signals (NLSs) of CaMKP-N were identified and characterized. CaMKP-N contains two NLSs, NLS1 and NLS2, at the C-terminus. A cluster of basic residues in the NLSs is important for their function. NLS1 and NLS2 function independently, but mutagenesis analysis suggests that these NLSs interact with each other.

Key words: CaM-kinase, CaMK phosphatase, CaMKP-N, nuclear localization signal, nuclear protein, protein kinase phosphatase, protein phosphatase.

Abbreviations: CaMK, calmodulin-dependent protein kinase; CaMKP, CaMK phosphatase; GFP, green fluorescent protein; NLS, nuclear localization signal; PBS, phosphate-buffered saline; PP, protein phosphatase.

Calmodulin-dependent protein kinases (CaMKs) are involved in various cellular reactions through a calcium stimulus (*1–3*). CaMKI (*4*, *5*) and CaMKIV (*6–8*) are activated through the phosphorylation of Thr^{177} and Thr^{199}, respectively, in their activation loops by CaMKK, and CaMKII (*9–12*) is activated through autophosphorylation of Thr^{286} within its autoinhibitory domain. We identified a protein phosphatase, CaMK phosphatase (CaMKP), which specifically dephosphorylates these phosphorylation sites of CaMKs (*13–15*). CaMKP is a calyculin A–insensitive, Mn^{2+}-dependent, and poly-L-lysine [poly(Lys)]–stimulated protein phosphatase. Rat CaMKP consists of 450 amino acid residues with a molecular weight of 49,165, which shows weak similarity (19.6%) to PP2Cα (*15*). Recently, we identified a CaMKP-related protein, CaMKP-N (*16*). Human CaMKP-N consists of 757 amino acid residues with a calculated molecular weight of 84,176 and is predominantly expressed in brain. Similarity (64%) between CaMKP-N and CaMKP was shown in the phosphatase domain and an adjacent N-terminal region. Although CaMKP-N and CaMKP belong to the PPM family (*17*), their relationships to other PPM members are weak. The enzymatic properties of CaMKP-N and CaMKP resemble each other, whereas their localizations are different. CaMKP-N is localized in the nucleus (*16*, *18*), whereas CaMKP is localized in the cytosol (*15*, *19*). Their substrates, CaMKs, also show specific distributions in cells. CaMKI is localized in the cytosol (*20*), but CaMKIV is localized in the nucleus (*21*, *22*). The subcellular localization of these proteins suggested the regulation of their activity through their localization. Therefore, the mechanism underlying their localization is intriguing. In the present study, the nuclear localization signals (NLSs) of CaMKP-N were analyzed. CaMKP-N contains two NLSs, NLS1 and NLS2, at the C-terminus. A cluster of basic residues in the NLSs is important for their function. NLS1 and NLS2 function independently, but mutagenesis analysis suggests that these NLSs interact with each other.

MATERIALS AND METHODS

Plasmid Construction—cDNA fragments of CaMKP-N were inserted into pEGFP-C1 or pEGFP-C3 (CLONTECH), in frame with the GFP coding sequences. For expression of CaMKP-N(1–757), a 2.3-kb *Xho*I–*Sal*I fragment of CaMKP-N cDNA, which encodes full-length CaMKP-N, was inserted into pEGFP-C1. For expression of C-terminal deletion mutants, *i.e.* CaMKP-N(1–69), CaMKP-N(1–152), CaMKP-N(1–269), and CaMKP-N(1–573), 0.2-kb *Xho*I–*Sma*I, 0.45-kb *Xho*I–*Pst*I, 0.8-kb *Xho*I–*Hin*dIII, and 1.7-kb *Xho*I–*Bgl*II fragments of CaMKP-N cDNA were inserted into pEGFP-C1, respectively. For expression of CaMKP-N(574–667), a 0.27-kb *Bgl*II–*Xba*I fragment of CaMKP-N cDNA was inserted into pEGFP-C3. For expression of CaMKP-N(668–757), a 0.5-kb *Xba*I-*Taq*I fragment of CaMKP-N cDNA was subcloned into pUC119, and the subcloned fragment was digested with *Bam*HI/*Hin*dIII and then inserted into pEGFP-C1. The other mutants were constructed by the method of Kunkel (*23*) or with a QuickChange site-directed mutagenesis kit (Stratagene) with appropriate synthetic oligonucleotides as primers. The mutations were confirmed by the dideoxynucleotide chain-termination method using a LI-COR model 4000L or an ABI Prism 310 DNA sequencer.

Cell Culture and Transfection—COS-7 cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum in a humidified incubator at 37°C

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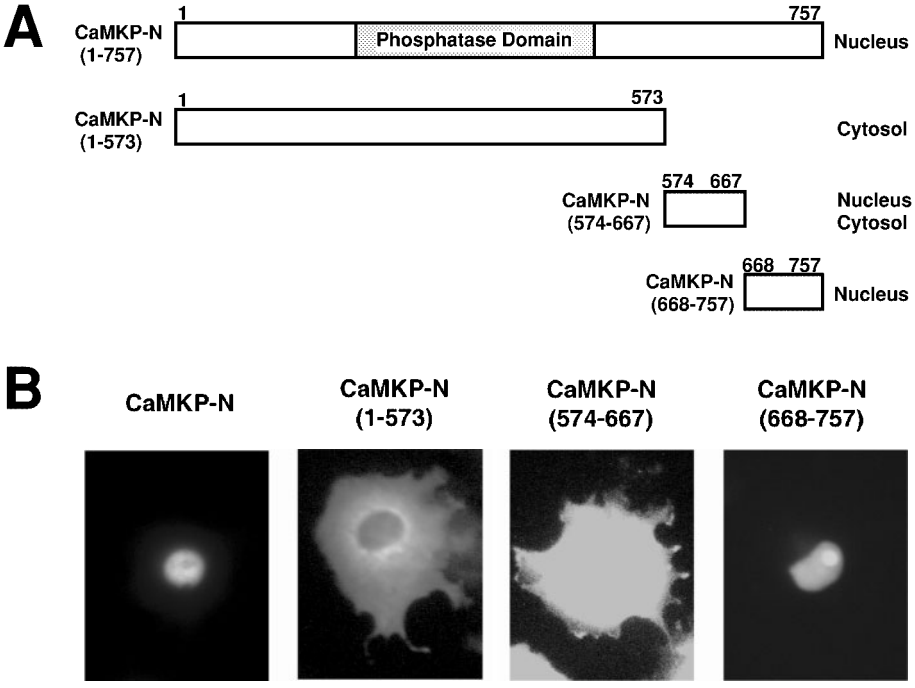

Fig. 1. **Localization of deletion mutants of CaMKP-N.** A: Schematic representation of the deletion constructs of CaMKP-N. A C-terminal deletion mutant, CaMKP-N(1–573), and other mutants, CaMKP-N(574–667) and CaMKP-N(668–757), were constructed. Expression vectors for the GFP-fusion proteins of the mutants were transfected into COS-7 cells, as described under "MATERIALS AND METHODS." B: Subcellular localization of the expressed proteins was visualized as the fluorescence of GFP. Data are representative of three independent experiments.

under an atmosphere of 5% CO_2. Transfection was carried out using Trans-IT-LT1 (PanVera Corp., Madison, WI), according to the manufacturer's instructions. In brief, COS-7 cells were plated at 1×10^5 cells/ml on a 60-mm dish in 3 ml of Dulbecco's modified Eagle's medium containing 10% fetal calf serum. After 20–24 h culture, the cells were incubated for 2–4 h in 3 ml of OPTI-MEM I Medium (Gibco BRL/Life Technologies) containing 6 μl of Trans-IT-LT1 and 3 μg of plasmid DNAs for transfection. After the incubation, the cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. Analysis was performed 1–2 d after transfection.

Fluorescence Microscopy—GFP-fusion proteins were detected in either living cells or fixed cells using a standard FITC filter set. Transfected COS-7 cells on coverslips were rinsed with phosphate-buffered saline (PBS), and then fixed with 4% formaldehyde for 30 min. After washing three times in PBS, the cells were mounted in Vectashield (Vector Laboratories), and then examined by fluorescence microscopy under a Zeiss Axioskop microscope (Carl Zeiss) equipped with a standard FITC filter set, 487909 (Carl Zeiss), using a Zeiss 40× Plan-NEOFLUOR lens (Carl Zeiss). For the examination of living cells, fluorescence microscopy was performed with a Zeiss Axiovert inverted microscope (Carl Zeiss) or an IX71 inverted microscope (Olympus).

RESULTS

Deletion Mutants of CaMKP-N—Comparison of the sequences of CaMKP-N and CaMKP revealed that CaMKP-N had large extra sequences at the N- and C-termini, which did not exist in CaMKP (*16*). This suggests that the NLS of CaMKP-N may exist at the N- or C-terminus. GFP-fusion vectors for various deletion mutants of CaMKP-N were constructed and transfected into COS-7 cells, and then the subcellular localization of the expressed proteins was determined from the fluorescence of GFP (Fig. 1). The full-length CaMKP-N fusion protein, CaMKP-N(1–757), was localized in the nucleus. In contrast, the C-terminal deletion mutant, CaMKP-N(1–573), was localized in the cytosol, and CaMKP-N(574–667) was localized in the whole cells. CaMKP-N(668–757), which contained the C-terminus, was localized in the nucleus. These results indicated that the NLS of CaMKP-N was localized in C-terminal residues 668–757. Other mutants, CaMKP-N(1–69) and CaMKP-N(1–152), which contained the N-terminus were localized in the whole cells, and CaMKP-N(1–269) was localized in the cytosol (data not shown). The localization of these proteins was probably dependent on their molecular size. Proteins smaller than about 40–60 kDa are able to enter the nucleus through diffusion, whrereas proteins larger than about 40–60 kDa can hardly enter the nucleus (24–26). These results indicated that the N-terminus region of CaMKP-N did not contain the NLS of CaMKP-N.

Deletion Mutants of CaMKP-N(668–757)—The C-terminal region, residues 668–757, in which the NLS was located, was analyzed in detail. Various deletion, from the N- or C-terminus, mutants of CaMKP-N(668–757) were constructed and analyzed (Fig. 2). CaMKP-N(668–702) and CaMKP-N(706–757) were localized in the nucleus. These mutants each contained distinctive regions. These results indicated that CaMKP-N contained two NLSs, not one. The two identified NLSs are designated as NLS1, which exists in residues 668–702, and NLS2, which exists in residues 706–757, and hereafter NLS1 and NLS2 are discussed separately.

Analysis of Mutants of NLS1—The region of NLS1 was analyzed in detail. CaMKP-N(668–696), the deletion being from the C-terminus of CaMKP-N(668–702), was localized in the whole cells. This result indicated that the C-terminal boundary of NLS1 was mapped between resi-

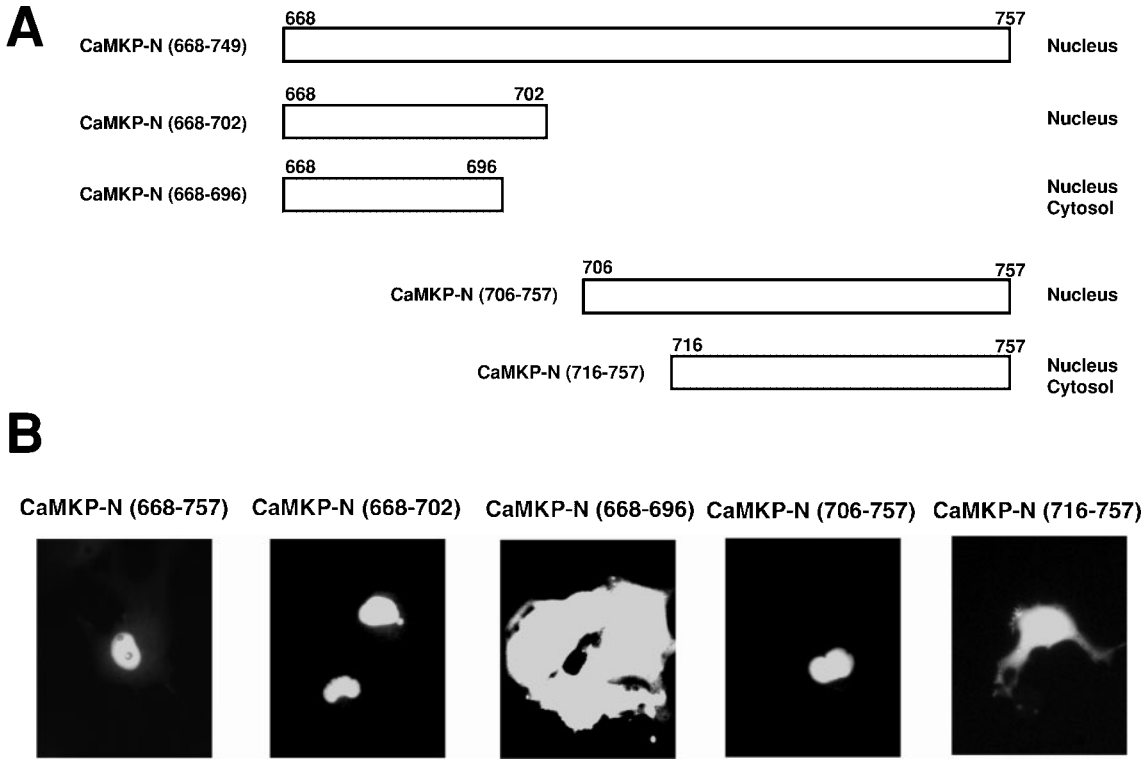

Fig. 2. **Localization of deletion mutants of CaMKP-N(668–757).** A: Schematic representation of the deletion mutants of CaMKP-N(668–757). C-terminal deletion mutants, CaMKP-N(668–702) and CaMKP-N(668–696), and N-terminal deletion mutants, CaMKP-N(706–757) and CaMKP-N(716–757), were constructed. Expression vectors for the GFP-fusion proteins of the mutants were transfected into COS-7 cells, as described under "MATERIALS AND METHODS." The results of subcellular localization of the mutants are indicated at the right. B: Subcellular localization of CaMKP-N(668–757), CaMKP-N(668–702), CaMKP-N(668–696) CaMKP-N(706–757), and CaMKP-N(716–757) is shown. Data are representative of three independent experiments.

dues 696 and 702 (Fig. 2). CaMKP-N(686–702), the deletion being from the N-terminus of CaMKP-N(668–702), was localized in the whole cells (Fig. 3). This result indicated that the N-terminal boundary of NLS1 was mapped between residues 668 and 686. It is known that a cluster of basic residues is important for classical NLSs (*24–26*). Two consecutive basic residues, Lys^{678}-Lys^{679}, exist in NLS1. An Ala substitution mutant, CaMKP-N(668–702)KK678AA, was constructed and transfected into COS-7 cells. CaMKP-N(668–702)KK678AA was local-

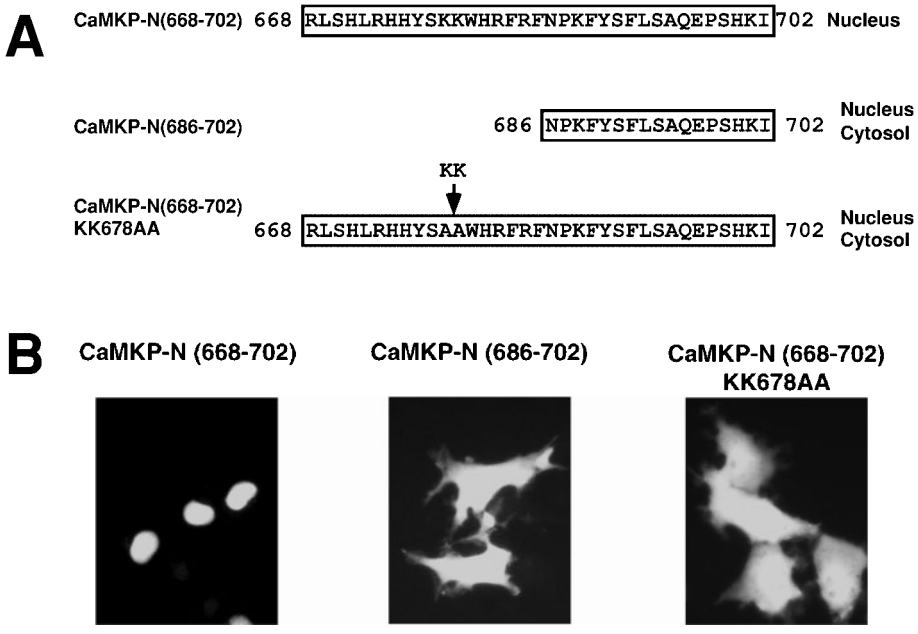

Fig. 3. **Analysis of mutants of NLS1.** A: Schematic representation of the mutants of CaMKP-N(668–702), which contains NLS1. An N-terminal deletion mutant, CaMKP-N(686–702), and an Ala substitution mutant, CaMKP-N(668–702)KK678AA, were constructed. Expression vectors for the GFP-fusion proteins of the mutants were transfected into COS-7 cells, as described under "MATERIALS AND METHODS." The results of subcellular localization of the mutants are indicated at the right. B: Subcellular localization of CaMKP-N(668–702), CaMKP-N(686–702), and CaMKP-N(668–702)KK678AA is shown. Data are representative of three independent experiments.

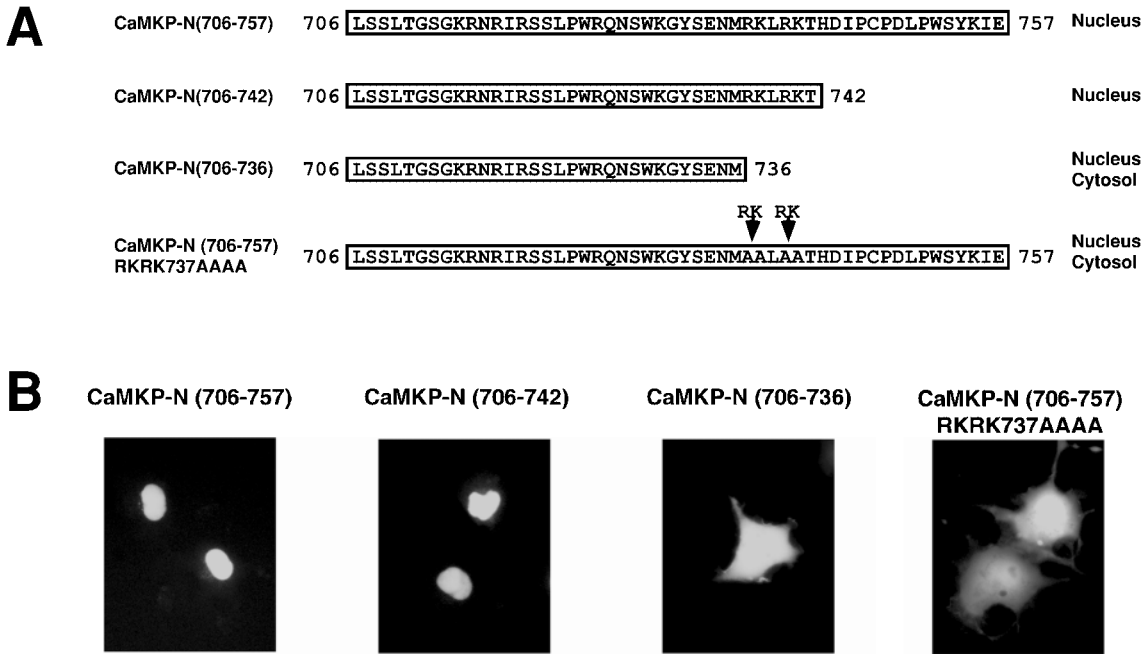

Fig. 4. **Analysis of mutants of NLS2.** A: Schematic representation of the mutants of CaMKP-N(706–757), which contains NLS2. C-terminal deletion mutants, CaMKP-N(706–742) and CaMKP-N(706–736), and an Ala substitution mutant, CaMKP-N(706–757)RKRK737AAAA, were constructed. Expression vectors for the GFP-fusion proteins of the mutants were transfected into COS-7 cells, as described under "MATERIALS AND METHODS." The results of subcellular localization of the mutants are indicated at the right. B: Subcellular localization of CaMKP-N(706–757), CaMKP-N(706–742), CaMKP-N(706–736), and CaMKP-N(706–757)RKRK737AAAA is shown. Data are representative of three independent experiments.

ized in the whole cells (Fig. 3). Thus, the basic residues Lys^{678}–Lys^{679} are important for the function of NLS1.

Analysis of Mutants of NLS2—The region of NLS2 was also analyzed in detail. CaMKP-N(716–757), the deletion being from the N-terminus of CaMKP-N(706–757), was localized in the whole cells (Fig. 2). This result indicated that the N-terminal boundary of NLS2 was mapped between residues 706 and 716. CaMKP-N(706–742), the deletion being from the C-terminus of CaMKP-N(706–757), was localized in the nucleus. In contrast, CaMKP-N(706–736) was localized in the whole cells (Fig. 4). These results indicated that the C-terminal boundary of NLS2 was mapped between residues 736 and 742. Interestingly, a cluster of basic residues, Arg^{737}–Lys^{738}, and Arg^{740}-Lys^{741}, exists in the region between residues 736 and 742. An Ala substitution mutant, CaMKP-N(706–757)RKRK737AAAA, was constructed and transfected into COS-7 cells. CaMKP-N(706–757)RKRK737AAAA was localized in the whole cells (Fig. 4). Thus, the cluster of basic residues, *i.e.* Arg^{737}–Lys^{738}, and Arg^{740}–Lys^{741}, is important for the function of NLS2.

Multi-Site Mutants of CaMKP-N(668–757)—Analysis of NLS1 and NLS2 separately revealed Ala substitution mutants that were unable to be functional NLSs. To verify that the combination of these mutations abolished the function of NLS in the consecutive fragment comprising residues 668–757, a multi-site mutant, CaMKP-N(668–757)K678,737A, which had the combined mutations of NLS1 and NLS2, CaMKP-N(668–702)KK678AA and CaMKP-N(706–757)RKRK737AAAA, was constructed and transfected into COS-7 cells. Surprisingly, CaMKP-N(668–757)K678,737A was localized in the nucleus (Fig. 5). Another Ala substitution mutant of NLS2, CaMKP-N(706–757)KR714AA, which was mutated at another cluster of basic residues, Lys^{714}–Arg^{715}, affected the localization of the mutant (data not shown). Therefore, a multi-site mutant, CaMKP-N(668–757)K678,714,737A, which included this mutation was constructed and transfected into COS-7 cells. CaMKP-N(668–757)K678, 714,737A was also localized in the nucleus. These results suggested that other residues in residues 668–757 are involved in the function of NLS. In the region of NLS1, there are two basic residues, Arg^{682} and Arg^{684}, near the basic residues Lys^{678}–Lys^{679}. Therefore, a multi-site mutant, CaMKP-N(668–757)K678,682,714,737A, was constructed and transfected into COS-7 cells. CaMKP-N(668–757)K678,682,714,737A was localized in the whole cells (Fig. 5). These results indicated that Arg^{682} and Arg^{684} are also involved in the function of NLS.

DISCUSSION

CaMKP-N and CaMKP constitute a family (*16*). CaMKP-N has large extra sequences at the N- and C-termini, which do not exist in CaMKP. Analysis of large deletion mutants of CaMKP-N revealed that the NLS of CaMKP-N existed in the C-terminus, residues 668–757 (Fig. 1). The mutants containing only the N-terminus of CaMKP-N were not localized in the nucleus (data not shown). These results indicate that the N-terminus of CaMKP-N does not function as an NLS. In the N-terminal region, some characteristic sequences exist, *i.e.* repeats of glutamate and proline residues, a cluster of glutamate residues, and a cluster of proline residues (*16*). The N-termi-

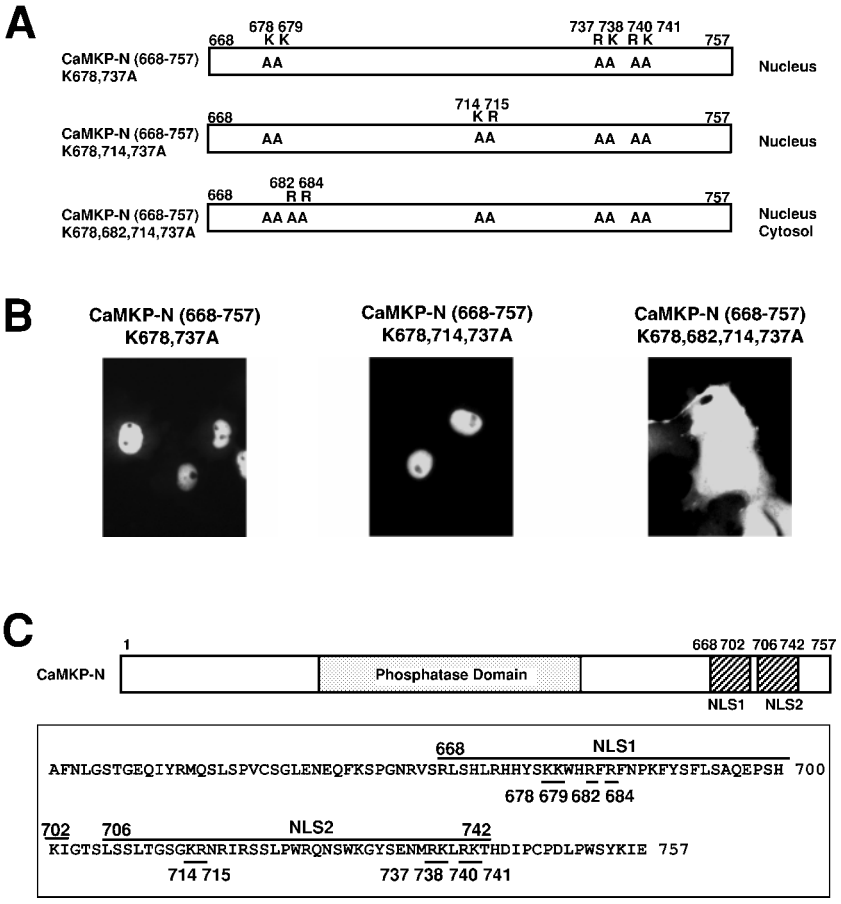

Fig. 5. **Localization of multi-site mutants of CaMKP-N(668–757).** A: Schematic representation of the multi-site mutants of CaMKP-N(668–757). Multi-site mutants, CaMKP-N(668–757)K678, 737A, CaMKP-N(668–757)K678, 714, 737A, and CaMKP-N(668–757)K678, 682, 714, 737A, were constructed. Expression vectors for the GFP-fusion proteins of the mutants were transfected into COS-7 cells, as described under "MATERIALS AND METHODS." B: Subcellular localization of CaMKP-N(668–757)K678, 737A, CaMKP-N(668–757)K678, 714, 737A, and CaMKP-N(668–757)K678, 682, 714, 737A is shown. Data are representative of three independent experiments. C: Schematic representation of the locations of the two NLSs of CaMKP-N. NLS1 consists of residues 668–702 and NLS2 consists of residues 706–742. The amino acid residues of which mutations affected the function of NLSs are indicated.

nus region might be involved in the activity through interaction with other proteins. Further studies are necessary to determine the function of the N-terminus of CaMKP-N.

Deletion analysis of the C-terminus region, residues 668–757, revealed that CaMKP-N contains two NLSs (Fig. 2), *i.e.* NLS1, which exists in residues 668–702, and NLS2, which exists in residues 706–757. Precise deletion mutant analysis revealed that NLS1 was mapped at residues 668–702 and NLS2 at residues 706–742 (Fig. 5C). The Ala substitution mutants, CaMKP-N(668–702)KK678AA, in which the basic residues, Lys^{678}–Lys^{679} were replaced, and CaMKP-N(706–757)RKRK737AAAA, in which the cluster of basic residues, Arg^{737}–Lys^{738}, and Arg^{740}–Lys^{741}, was replaced, were unable to function as NLSs (Figs. 3 and 4). The well known classical NLS motifs consist of monopartite and bipartite NLSs (*24–26*). In these classical NLSs, a cluster of basic residues is important. In this regard, due to the importance of basic residues, the NLSs of CaMKP-N may belong to the classical NLS motifs. But there is a difference in that the NLSs of CaMKP-N relatively long sequences and the classical NLSs consist of short sequences. Further studies are necessary to classify the NLSs of CaMKP-N. Classical NLSs bind importin α (*27–30*). Therefore the NLSs of CaMKP-N may bind importin α.

Curiously, the combination of the Ala substitution mutations of NLS1 and NLS2 did not disturb their localization, although these mutations abolished the NLS function when the region was divided into two parts (Fig. 5). This result was not due to the usage of the fragment, since the full-length mutant, CaMKP-N-K678,714,737A, was also localized in the nucleus (data not shown). Introduction of additional mutations at the residues, Arg^{682} and Arg^{684}, near the basic residues, Lys^{678}–Lys^{679}, led to loss of the NLS function. These results suggest that NLS1 and NLS2 function independently, but that they may interact with each other. Although there are no detectable repetitive sequences, NLS1 and NLS2 may constitute a large domain, which is composed of multiple interaction sites, like fingers. The crystal structure of CaMKP-N may be necessary to solve this problem.

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REFERENCES

1. Braun, A.P. and Schulman, H. (1995) The multifunctional calcium/calmodulin-dependent protein kinase: from form to function. *Annu. Rev. Physiol.* **57**, 417–445
2. Heist, E.K. and Schulman, H. (1998) The role of Ca^{2+}/calmodulin-dependent protein kinases within the nucleus. *Cell Calcium* **23**, 103–114
3. Soderling, T.R. (1999) The Ca^{2+}-calmodulin-dependent protein kinase cascade. *Trends Biochem. Sci.* **24**, 232–236
4. Mochizuki, H., Sugita, R., Ito, T., and Hidaka, H. (1993) Phosphorylation of Ca^{2+}/calmodulin-dependent protein kinase V

and regulation of its activity. *Biochem. Biophys. Res. Commun.* **197**, 1595–1600
5. Lee, J.C. and Edelman, A.M. (1994) A protein activator of Ca^{2+}-calmodulin-dependent protein kinase Ia. *J. Biol. Chem.* **269**, 2158–2164
6. Okuno, S. and Fujisawa, H. (1993) Requirement of brain extract for the activity of brain calmodulin-dependent protein kinase IV expressed in *Escherichia coli*. *J. Biochem.* **114**, 167–170
7. Okuno, S., Kitani, T., and Fujisawa, H. (1994) Purification and characterization of Ca^{2+}/calmodulin-dependent protein kinase IV kinase from rat brain. *J. Biochem.* **116**, 923–930
8. Tokumitsu, H., Brickey, D.A., Glod, J., Hidaka, H., Sikela, J., and Soderling, T.R. (1994) Activation mechanisms for Ca^{2+}/calmodulin-dependent protein kinase IV. Identification of a brain CaM-kinase IV kinase. *J. Biol. Chem.* **269**, 28640–28647
9. Kwiatkowski, A.P., Shell, D.J., and King, M.M. (1988) The role of autophosphorylation in activation of the type II calmodulin-dependent protein kinase. *J. Biol. Chem.* **263**, 6484–6486
10. Katoh, T. and Fujisawa, H. (1991) Autoactivation of calmodulin-dependent protein kinase II by autophosphorylation. *J. Biol. Chem.* **266**, 3039–3044
11. Ikeda, A., Okuno, S., and Fujisawa, H. (1991) Studies on the generation of Ca^{2+}/calmodulin-independent activity of calmodulin-dependent protein kinase II by autophosphorylation. Autothiophosphorylation of the enzyme. *J. Biol. Chem.* **266**, 11582–11588
12. Ishida, A., Kitani, T., and Fujisawa, H. (1996) Evidence that autophosphorylation at Thr-286/Thr-287 is required for full activation of calmodulin-dependent protein kinase II. *Biochim. Biophys. Acta.* **1311**, 211–217
13. Ishida, A., Kameshita, I., and Fujisawa, H. (1998) A novel protein phosphatase that dephosphorylates and regulates Ca^{2+}/calmodulin–dependent protein kinase II. *J. Biol. Chem.* **273**, 1904–1910
14. Ishida, A., Okuno, S., Kitani, T., Kameshita, I., and Fujisawa, H. (1998) Regulation of multifunctional Ca^{2+}/calmodulin–dependent protein kinases by Ca^{2+}/calmodulin–dependent protein kinase phosphatase. *Biochem. Biophys. Res. Commun.* **253**, 159–163
15. Kitani, T., Ishida, A., Okuno, S., Takeuchi, M., Kameshita, I., and Fujisawa, H. (1999) Molecular cloning of Ca^{2+}/calmodulin–dependent protein kinase phosphatase. *J. Biochem.* **125**, 1022–1028
16. Takeuchi, M., Ishida, A., Kameshita, I., Kitani, T., Okuno, S., and Fujisawa, H. (2001) Identification and characterization of CaMKP-N, nuclear calmodulin–dependent protein kinase phosphatase. *J. Biochem.* **130**, 833–840
17. Cohen, P. (1989) The structure and regulation of protein phosphatases. *Annu. Rev. Biochem.* **58**, 453–508
18. Kitani, T., Okuno, S., Takeuchi, M., and Fujisawa, H. (2003) Subcellular distributions of rat CaM kinase phosphatase N and other members of the CaM kinase regulatory system. *J. Neurochem.* **86**, 77–85
19. Nakamura, Y., Kitani, T., Okuno, S., Otake, K., Sato, F., and Fujisawa, H. (2000) Immunohistochemical study of the distribution of Ca^{2+}/calmodulin–dependent protein kinase phosphatase in the rat central nervous system. *Mol. Brain Res.* **77**, 76–94
20. Picciotto, M.R., Zoli, M., Bertuzzi, G., and Nairn, A.C. (1995) Immunochemical localization of calcium/calmodulin–dependent protein kinase I. *Synapse* **20**, 75–84
21. Jensen, K.F., Ohmstede, C.A., Fisher, R.S., and Sahyoun, N. (1991) Nuclear and axonal localization of Ca^{2+}/calmodulin–dependent protein kinase type Gr in rat cerebellar cortex. *Proc. Natl Acad. Sci. USA* **88**, 2850–2853
22. Nakamura, Y., Okuno, S., Sato, F., and Fujisawa, H. (1995) An immunohistochemical study of Ca^{2+}/calmodulin–dependent protein kinase IV in the rat central nervous system: light and electron microscopic observations. *Neuroscience* **68**, 181–194
23. Kunkel, T.A. (1985) Rapid and efficient site-specific mutagenesis without phenotypic selection. *Proc. Natl Acad. Sci. USA* **82**, 488–492
24. Dingwall, C. and Laskey, R.A. (1991) Nuclear targeting sequences—a consensus? *Trends Biochem. Sci.* **16**, 478–481
25. Mattaj, I.W. and Englmeier, L. (1998) Nucleocytoplasmic transport: the soluble phase. *Annu. Rev. Biochem.* **67**, 265–306
26. Görlich, D. and Kutay, U. (1999) Transport between the cell nucleus and the cytoplasm. *Annu. Rev. Cell. Dev. Biol.* **15**, 607–660
27. Conti, E. and Kuriyan, J. (2000) Crystallographic analysis of the specific yet versatile recognition of distinct nuclear localization signals by karyopherin α. *Structure* **8**, 329–338
28. Conti, E., Uy, M., Leighton, L., Blobel, G., and Kuriyan, J. (1998) Crystallographic analysis of the recognition of a nuclear localization signal by the nuclear import factor karyopherin α. *Cell* **94**, 193–204
29. Fontes, M.R., Teh, T., and Kobe, B. (2000) Structural basis of recognition of monopartite and bipartite nuclear localization sequences by mammalian importin-α. *J. Mol. Biol.* **297**, 1183–1194
30. Kobe, B. (1999) Autoinhibition by an internal nuclear localization signal revealed by the crystal structure of mammalian importin α. *Nat. Struct. Biol.* **6**, 388–397