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Tac2-N, an atypical C-type tandem C2 protein localized in the nucleus[1]

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The C2 domain was first identified as a Ca^{2+}-binding module homologous to the C2 regulatory region of mammalian Ca^{2+}-dependent protein kinase C isoforms and is found in various signaling molecules and proteins involved in vesicular trafficking (reviewed in [1]). C2 domains are composed of a common eight-stranded anti-parallel β-sandwich consisting of four-stranded β-sheets. Three flexible loops are formed at the tip of the β-sandwich structure, and contain conserved negatively charged amino acids (Asp or Glu) that are essential for Ca^{2+}-binding [1–3]. Ca^{2+}-binding triggers interaction of the C2 domain with phospholipids, thereby enabling translocation of proteins to the phospholipid membrane where they function [1].

The role of the C2 domain is not limited to Ca^{2+}-dependent phospholipid binding, since this motif has been shown to have a variety of functions in cellular signaling. For instance, the synaptotagmin I (Syt I) C2 domain, one of the best characterized C2 domains essential for neurotransmitter release, functions as a Ca^{2+}-dependent and -independent protein-interaction site in addition to being a phospholipid-binding site [4]. Very recently, we showed that the C2A domain of Doc2γ, a third isoform of double C2 protein [5], contains a functional nuclear-localization signal [6]. The nuclear-localization signal of the C2A domain of Doc2γ has been mapped to a six Arg cluster in the loop between the β6 and β7 strands by mutation and deletion analyses (underlined in Fig. 1A). In conventional C2 domains, this is the third Ca^{2+}-binding loop, but because the coordinating Glu and Asp residues have been mutated, Doc2γ is incapable of Ca^{2+}-binding (asterisks in Fig. 1A) [5,6].

This finding raised a question as to whether some Ca^{2+}-independent C2 domains function as a nuclear-localization signal by changing the nature of Ca^{2+}-binding loops, or the Doc2γ C2A domain is the sole C2 domain involved in nuclear localization. To address this issue, we searched nucleotide and protein databases to identify novel C2 domains that have a basic cluster (more than three serial Lys or Arg residues), but lack negatively charged amino acids (Glu or Asp) responsible for Ca^{2+}-binding. We found one putative C2 domain-containing protein and cloned the mouse cDNA containing the open reading frame (encoding 489 amino acids) by reverse-transcriptase-polymerase chain reaction, using standard methods [5]. Since this putative protein contains a C2A domain, a C2B domain, and a short C-terminus including a WHXL motif [7], the protein should be classified into the carboxyl-terminal-type (C-type) tandem C2 protein family [8], but is distinguished from other tandem C2 protein families (e.g. synaptotagmins, synaptotagmin-like proteins (Slps), and Doc2s) in that it has no transmembrane domain, Slp-homology domain or Munc13-1-interacting domain at the N-terminus. Homology search analysis revealed that the N-terminal domain of this protein contains no known protein motifs. Phylogenetic analysis also indicated that this protein represents a novel class of C-type tandem C2 proteins (data not shown). Thus, we named this protein Tac2-N (tandem C2 protein in nucleus; see below).

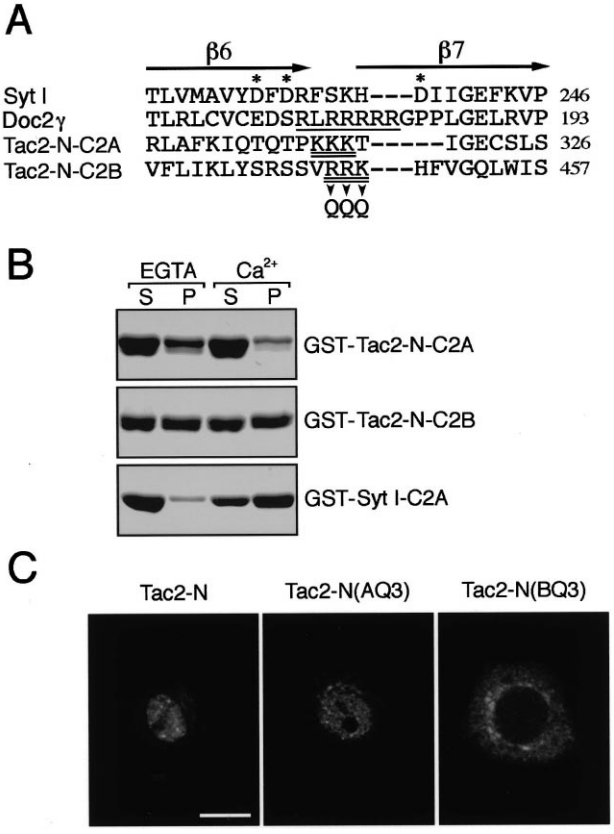

Fig. 1. A basic cluster in the C2B domain is essential for nuclear localization of Tac2-N. A: Alignment of the third putative Ca^{2+}-binding loops of the C2 domains of Syt I, Doc2γ, and Tac2-N. Asterisks indicate Asp and/or Glu residues, which are crucial for Ca^{2+}-binding in the Syt I–C2A domain [1]. The single and double underlines indicate the basic clusters of Doc2γ (six Arg) and Tac2-N (KKK in the C2A domain and RRK in the C2B domain), respectively. The locations of β-strands are indicated by arrows [2]. The arrowheads indicate RRK-to-QQQ substitution (BQ3 mutation of Tac2-N). Amino acid numbers are indicated on the right. B: Ca^{2+}-dependent phospholipid-binding properties of Tac2-N C2 domains. Phosphatidylserine (PS)/phosphatidylcholine liposomes and glutathione *S*-transferase (GST)-fusion proteins were incubated in 50 mM HEPES–KOH, pH 7.2, in the presence of 2 mM EGTA or 1 mM Ca^{2+} for 15 min at room temperature. After centrifugation at $12\,000\times g$ for 10 min, the supernatants (S, non-binding fraction) and pellets (P, phospholipid-binding fraction) were separated as described previously [6]. Equal proportions of the supernatants and pellets were subjected to 10% SDS–polyacrylamide gel electrophoresis and then stained with Coomassie brilliant blue R-250. C: Subcellular localizations of T7-Tac2-N, -Tac2-N(AQ3) and -Tac2-N(BQ3). PC12 cells expressing pEF-T7-Tac2-N were fixed, permeabilized, and stained with anti-T7 tag antibody as described previously [6]. Note that the neutralization of the basic sequence (RRK) in the Tac2-N C2B domain is sufficient to abolish nuclear localization. Scale bar indicates 10 μm.

Sequence alignment of the third putative Ca^{2+}-binding loop of the C2A domain of Syt I, Doc2γ and two C2 domains of Tac2-N suggests that Tac2-N has three serial basic residues in the third loop of each C2 domain (KKK in the C2A domain and RRK in the C2B domain; double underlines in Fig. 1A), in approximately the same location as the Arg cluster of the Doc2γ C2A domain. By contrast, the C2 domains of other C-type tandem C2 proteins mainly localized outside the nucleus contain only one or two basic residues in the corresponding location (data not shown). Since the C2 domain of Tac2-N lacks Asp or Glu residues responsible for Ca^{2+}-binding, Tac2-N should be classified as a Ca^{2+}-independent type-C2 domain. As expected, the C2A domain of Tac2-N did not show Ca^{2+}-dependent phospholipid-binding activity (phosphatidylserine/phosphatidylcholine (PC) liposome; 1:1, w/w) and the C2B domain of Tac2-N weakly bound liposomes irrespective of the presence of Ca^{2+} (Fig. 1B, top and middle panels) [5,6]. By contrast, the C2A domain of Syt I bound liposomes only in the presence of Ca^{2+} (Fig. 1B, bottom panel).

To further examine whether Tac2-N is indeed localized in the nucleus, T7-tagged Tac2-N was expressed in PC12 cells (Fig. 1C, left panel). As expected, Tac2-N proteins were almost exclusively localized in the nucleus, like Doc2γ proteins [6]. Neutralization of basic residues (mutation to Gln) in the C2A domain (AQ3) could not prevent the nuclear localization of Tac2-N (Fig. 1C, middle panel), while neutralization of basic residues in the C2B domain (BQ3) resulted in cytosolic and perinuclear localizations of mutant proteins (Fig. 1C, right panel). These results indicate that the basic cluster (RRK) in the third loop of the C2B domain is essential for nuclear localization of Tac2-N protein, while a similar basic cluster (KKK) in the C2A domain is not involved in nuclear localization. By contrast, Doc2γ protein contains a nuclear-localization signal in the C2A, but not the C2B domain [6]. Based on these results, we propose that, at least, several C2 domains function as nuclear-localization signals rather than Ca^{2+}-sensors, by changing the nature of the Ca^{2+}-binding loop. Completion of the human and mouse genomes is anticipated to uncover more C2 domain-containing proteins that may function in the nucleus.

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[1] The nucleotide sequence reported in this paper is deposited in the DDBJ, EMBL, and GenBank nucleotide sequence databases with accession number of AB062282.

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