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Minireview

CAMTAs: Calmodulin-binding transcription activators from plants to human

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Abstract **Recently, a novel family of calmodulin-binding transcription activators (CAMTAs) was reported in various eukaryotes. All CAMTAs share a similar domain organization, with a novel type of sequence-specific DNA-binding domain (designated CG-1). This domain could bind DNA directly and activate transcription, or interact with other transcription factors, not through DNA binding, thus acting as a co-activator of transcription. Investigations of CAMTAs in various organisms imply a broad range of functions from sensory mechanisms to embryo development and growth control, highlighted by the apparent involvement of mammalian CAMTA2 in cardiac growth, and of CAMTA1 in tumor suppression and memory performance.**

Keywords: Calcium; Calmodulin; Hypertrophy; Signal transduction; Transcription factor; Tumor suppression

1. Introduction

Transcription factors (TFs) play a crucial role in regulating every aspect of the organism's life cycle and are fit to respond to signals originating from within and without the organism. Ca^{2+} plays a key role in regulating gene transcription [1]. The mechanisms of Ca^{2+}-dependent transcription regulation are numerous and include various signal transducers, such as the superfamily of EF-hand Ca^{2+}-binding proteins (e.g. calmodulin, CaM) [1]. These regulate the activity of a number of transcriptional regulators such as the cAMP transcriptional activator CREB and its versatile co-activator CREB-binding protein CBP300 [2]. The expression of the mammalian *c-fos* gene is mediated by Ca^{2+} signals through two DNA regulatory elements, the CRE (cyclic-AMP-response element) and the SRE (serum-response element). Increase in nuclear Ca^{2+} concentration stimulates CRE-dependent gene expression, whereas elevation of cytosolic Ca^{2+} activates transcription via SRE [3]. Likewise, in plants different sets of genes are regulated by cytosolic and nuclear Ca^{2+} signals [4]. Thus, nuclear and cytoplasmic Ca^{2+} signals control transcription by distinct mechanisms. Ca^{2+} can also directly bind to and regulate certain TFs. The DREAM protein contains four EF-hand motifs and represses transcription [5], as DREAM affinity for DNA is reduced upon binding to Ca^{2+}. Similarly, a basic helix–loop–helix (bHLH) TF (AtNIG1) involved in salt-stress signaling in plants was also reported to directly bind Ca^{2+} [6]. In addition, certain TFs of the bHLH family were shown to directly bind CaM, thus inhibiting DNA-binding by masking the DNA-binding domain [7–9]. In plants, recent reports suggest the occurrence of other types of CaM-binding TFs including WRKY [10], Myb [11], and Calmodulin-binding Transcription Activators (CAMTAs) [12].

2. CAMTAs′ domain organization

The CAMTA proteins consist of multiple predicted functional domains, evolutionarily conserved in amino acid sequences, and organized in a conserved order (Fig. 1). The functional domains include: nuclear localization signals (NLS); CG-1, a unique DNA-binding domain (see details below); TIG, a domain implicated in nonspecific DNA contacts in TFs [13], and involved in protein dimerization [14,15]; ANK (ankyrin) repeats, which are present as tandemly repeated modules of about 33 amino acids in a large number of eukaryote proteins and viruses, and participate in protein–protein interactions [16–18]. In addition, CAMTAs contain a variable number of IQ motifs [12]. The IQ motifs consist of low complexity regions with the repetitive motif IQXXXRGXXX and are known to be associated with binding of CaM and CaM-like proteins [19,20]. Recent investigations in fly, mammals and plants, confirm the function of these domains in controlling gene expression, however, with interesting variations. Mapping of a Ca^{2+}-dependent CaM-binding domain in *Arabidopsis* AtCAMTA1 revealed a single high-affinity binding site ($Kd \sim 1.2$ nM) within an 18-amino acid region adjacent to the IQ motifs [12], predicting the occurrence of multiple CaM-binding sites with complex regulatory properties. Analysis of a rice CAMTA revealed a Ca^{2+}-dependent CaM-binding domain and 4 Ca^{2+}-dependent CaM-dissociation domains, equivalent to the IQ motifs (Ca^{2+}-independent), localized in the C-terminus [21]. Analysis of transcription regulation by a rice CAMTA using a synthetic promoter revealed that Ca^{2+}/CaM inhibited CAMTA-mediated transcription. In contrast, in *Drosophila* a Ca^{2+}-independent binding site for CaM was found within an IQ motif, and CaM binding to DmCAMTA is a prerequisite for DmCAMTA transcriptional activity [22]. CaM activation of DmCAMTA, however, is controlled by Ca^{2+} as evident in

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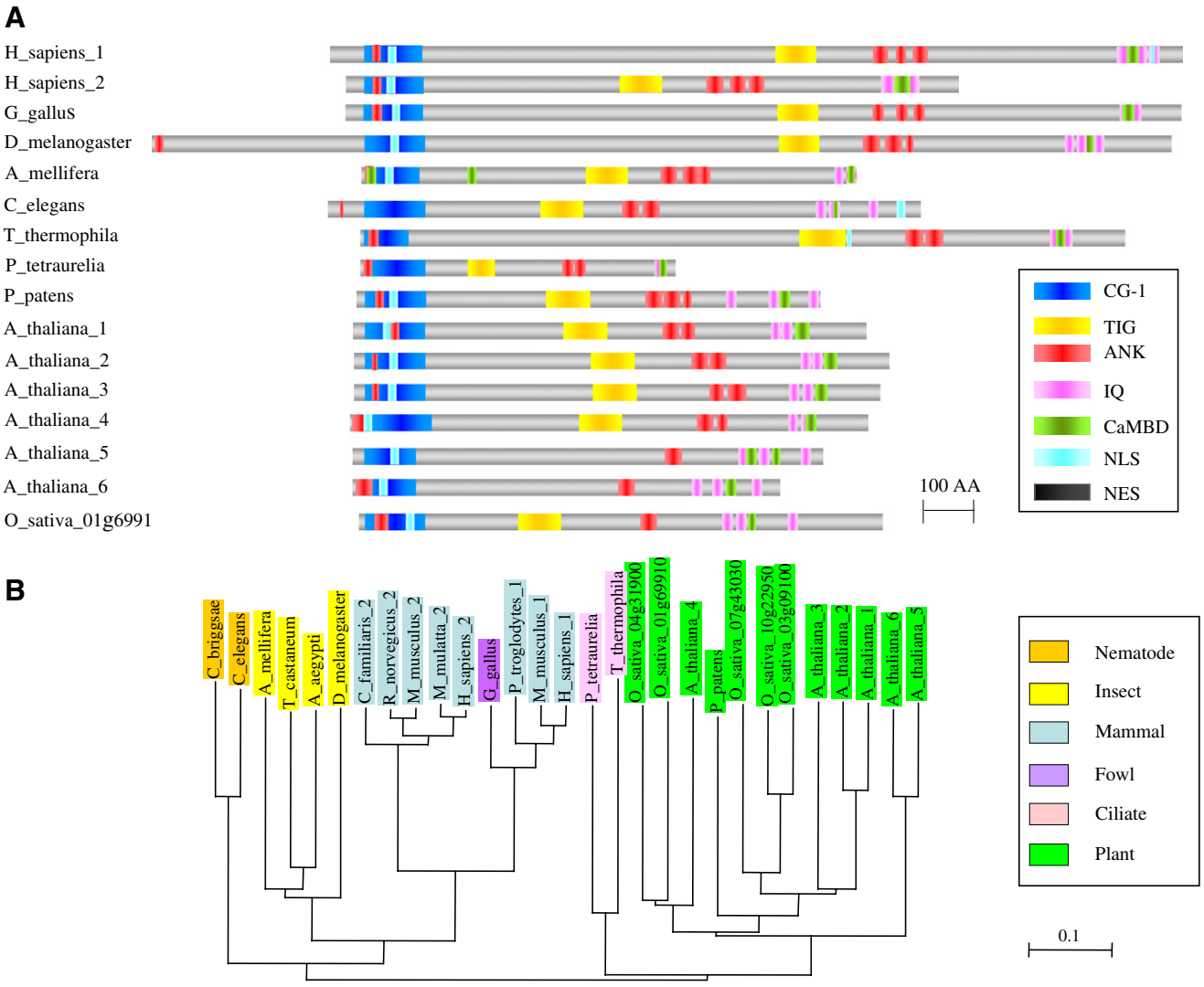

Fig. 1. Bioinformatics analysis of CAMTAs' domain organization, and phylogeny. (A) Domain organization: presentation of CAMTAs (drawn to scale) from multicellular and unicellular eukaryotes was obtained by NCBI/BLAST/CDART (Conserved Domain Architecture Retrieval Tool) at http://www.ncbi.nlm.nih.gov/Structure/lexington/lexington.cgi, based on NCBI http://www.ncbi.nlm.nih.gov/Entrez and http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi, Pfam (http://www.sanger.ac.uk/Software/Pfam) and DOUTfinder (http://mendel.imp.ac.at/dout/). CaM-binding domains (CaMBD) were specifically searched at http://calcium.uhnres.utoronto.ca/ctdb/flash.htm; Nuclear localization signals (NLS) were searched by a few programs: PredictNLS at http://cubic.bioc.columbia.edu/cgi/var/nair/resonline.pl, which searches for monopartite NLSs, exemplified by the SV40 large T antigen NLS (PKKKRRV), and bipartite NLSs, exemplified by the nucleoplasmin NLS (KRPAATKKAGQAKKKK); Motifscan at http://myhits.isb-sib.ch/cgi-bin/motif_scan; and the PSORT at http://www.psort.org/. Nuclear export signals (NES) were searched at http://www.cbs.dtu.dk/services/NetNES/. Transcription activation domains (TADs) were experimentally mapped to a region between the CG-1 and a transcription factor immunoglobulin (TIG)-like DNA-binding domain, domains in both AtCAMTA1 [12] and HsCAMTA2 [18], but as these could not be identified by bioinformatics analysis, they are not shown in Fig. 2. The CG-1 domain interacts with DNA *cis*-elements as described. In addition, the CG-1 domain of HsCAMTA2 was found to interact with the homeodomain of the Nkx2.5 TF (see Fig. 2), and acts as co-activator of transcription. Using the Superfamily bioinformatics program (http://supfam.mrc-lmb.cam.ac.uk/SUPERFAMILY/hmm.html), a previously unrecognized ANK domain was found within the CG-1 domain, suggesting a role for the CG-1 domain in protein-protein interactions. In fact, N-terminus ANK domains are found in almost all CAMTAs (except AtCAMTA5), suggesting that CAMTAs participate in multi-component complexes. The NLS were deduced by at least one search algorithm. NLS are localized to the N-terminus in most CAMTAs, with exceptions in some rice CAMTAs, which have an additional NLS at the C-terminus, as confirmed experimentally [21]. NLS is not detected in all CAMTAs: *Paramecium* CAMTA lacks an apparent NLS, but contains a putative NES in the N-terminus. In *C. elegans*, CAMTA contains both NLS and NES, localized to the C- and N-termini, respectively. Bioinformatics analysis of HsCAMTA2 detected NLS only in the N-terminus, but experimental evidence localized the NLS to the C-terminus, and an NES to the N-terminus [18]. (B) Phylogram tree: the tree was constructed using ClustalW (http://www.ebi.ac.uk/clustalw/), colored by phylogenetic classification: Nematodes, metallic gold; Insects, yellow; Mammals, light blue; Fowl, purple; Ciliates (unicellular protozoa), pink; Plants (monocotyledons, dicotyledons, and moss), green. CAMTA accession numbers:*Homo sapiens:* HsCAMTA1 (Q9Y6Y1), HsCAMTA2 (O94983); *Mus musculus*: CAMTA1 (CAM18835), CAMTA2 (CAM28144); *Gallus gallus*, red jungle fowl (XP_417530); *Pan troglodytes*, chimpanzee (XP_514346); *Canis familiaris*, dog (XP_546572); *Rattus norvegicus*, Norway rat (XP_213362); *Macaca mulatta*, rhesus monkey (XP_001117780); *Drosophila melanogaster*, fruit fly (ABI94369); *Apis mellifera*, honey bee (XP_001120489); *Aedes aegypti*, yellow fever musquito (EAT45641); *Tribolium castaneum*, red flour beetle (XP_968552);*Tetrahymena thermophila*, ciliate protozoa (XP_001011181); *Paramecium tetraurelia*, unicellular ciliate protozoa (CAK81933);*Caenorhabditis elegans*, nematode (NP_494796); *Caenorhabditis briggsae* (CAE67879); *Physcomitrella patens*, moss (gw1.188.72.1); *Arabidopsis thaliana*: AtCAMTA1 (Q9FY74), AtCAMTA2 (Q6NPP4), AtCAMTA3 (Q8GSA7), AtCAMTA4 (NP_176899), AtCAMTA5 (O23463), AtCAMTA6 (NM_112570); *Oryza sativa*: Os01g69910, Os03g09100, Os04g31900, Os07g43030, Os10g22950. The scale bar represents the number of changes per site.