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BRIEF COMMUNICATION

Microtubule interaction of LICC1, a maize homologue of a component of the human muskelin/RanBPM/CTLH protein complex

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Abstract

MRCTLH (muskelin/RanBPM/CTLH) is a protein complex found in humans (MRCTLH) that is involved in the regulation of numerous cellular processes, such as gluconeogenesis, cell signaling, development, nuclear extrusion, cell morphology, or stability of different proteins. According to genomic data, all eukaryotes have similar protein complexes. In yeast, a similar protein complex named GID was found to be involved in the regulation of gluconeogenesis. LICC1 is a maize protein whose sequence resembles that of TWA1 in humans and GID8 in yeast, which are central components of the MRCTLH and GID complexes. LICC1 contains three highly conserved protein domains, LisH, CTLH, and CRA, typical of this protein family. *Twa1* and *gid8* are unique genes in human and yeast genomes. However, three copies of *licc1* are present in the maize genome and multiple copies are present in other plant genomes. This result suggests the presence of multiple variants of the MRCTLH/GID complex in plants, which could increase its regulatory capacity. We also demonstrate here that LICC1 has the ability to interact with microtubules, similarly to the human TWA1. This interaction reinforces the idea that the LICC1 protein from maize, and its homologues in plants and, in general, the GID/MRCTLH complex in plants, can perform biological functions similar to those in humans and yeast.

Keywords: GID, GID8, LisH-CTLH-CRA, MRCTLH, TWA1.

Yeast GID is a 600 kDa protein complex composed of seven proteins that functions as a ubiquitin-ligase complex and participates in the inactivation of gluconeogenesis (Menssen *et al.* 2012). There are GID-like protein complexes in most Eukaryotes, but not in Prokaryotes (Francis *et al.* 2013). In humans, the complex is called MRCTLH, from muskelin/RanBP9/CTLH, and is composed of eight proteins that are homologous in domain organization and sequence identity to yeast GID proteins (Maitland *et al.* 2019). Human MRCTLH also has E3 ubiquitin ligase activity and is involved in various processes including cell signaling (Salemi *et al.* 2017), development (Yoo *et al.* 2017), nuclear extrusion (Soni *et al.* 2006), regulation of cell morphology (Valiyaveettil *et al.* 2008) or regulation of the stability of different proteins (Suresh *et al.* 2010).

Importantly, many of the proteins that form these complexes contain contiguous domains of lissencephaly-1 homology (LisH), C-terminal to LisH (CTLH), and C-terminal CT11-RanBP9 (CRA). LisH participates in homodimerization and in determining the cellular localization of proteins (Emes and Ponting 2001). The exact role of the CTLH domain is still unknown, but it appears to have α-helical structure, which is assumed to be involved in protein-protein interactions (Umeda *et al.* 2003). The CRA domain also represents a protein-protein interaction domain (Menon *et al.* 2004).

In plants, different genes have been found to encode proteins showing sequence similarity with the components

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Abbreviations: CTLH - C-terminal LisH motif; CRA - CT11_RanBPM domain; GID - glucose-induced degradation complex; LICC1 - LisH-CTLH-CRA 1 protein; LisH - LIS1 homology domain; MRCTLH - muskelin/RanBP9/CTLH complex.
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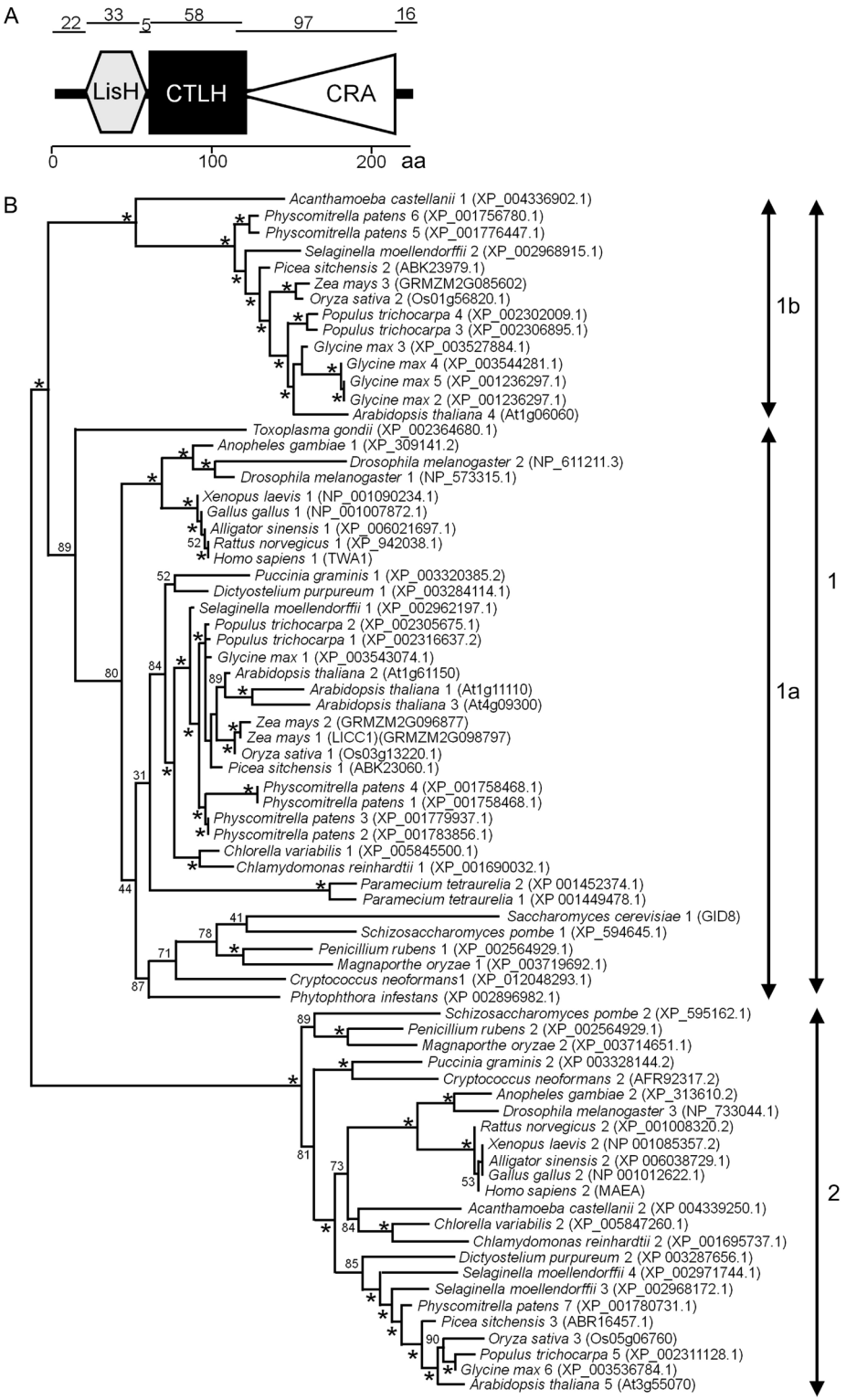

Fig. 1. *A* - Schematic representation of protein LICC1 including the three conserved domains LisH, CTLH, and CRA. The sizes of each domain are indicated in the top (amino acids). Protein motifs were identified using *SMART* (http://smart.embl-heidelberg.de/). *B* - Unrooted maximum-likelihood tree based on the sequences of proteins from different species that contain the three conserved protein domains LisH, CTLH, and CRA. References of the sequences are indicated. Only the positions that are conserved in all the sequences were used in this analysis. The sequence of LICC1 protein was used as a query for *BLASP* and *TBLASTN*. Amino acid sequences were aligned using *ClustalW* (https://npsa-prabi.ibcp.fr/NPSA/npsa_clustalw.html). Phylogenetic tree was constructed by maximum likelihood using *IQ-TREE* (http://iqtree.cibiv.univie.ac.at/) with 1 000 iterations. Bootstrap values higher than 90 are shown as an *asterisk*.

of the GID/MRCTLH complexes. In *Arabidopsis thaliana*, interactions between some of these proteins have been demonstrated (Tomaštíková *et al*. 2012, Miquel and Vicient 2017), reinforcing the idea that the GID/MRCTLH protein complex is also present in plants (Francis *et al*. 2013). However, a proper knowledge of their structure and functions in plants is still lacking.

TWA1 and GID8 are the smallest protein components of these complexes and they act as scaffolds for the other components (Francis *et al*. 2017). TWA1 and GID8 contain the LisH, CTLH, and CRA domains. In yeast, the overexpression of GID8 accelerates DNA replication**,** whereas the absence of *gid8* produces a delay of DNA replication as well as an increase of cell size (Pathak *et al*. 2004). In humans, the TWA1 protein can be localized in the cytoplasm and in the nucleus, and in Hela cells, it co-localizes with the α-tubulin (Lu *et al*. 2017, Salemi *et al*. 2017). TWA1 promotes the nuclear retention of some proteins such as β-catenin (a protein involved in stem cell renewal and organ regeneration), and is essential in the dorsal development of zebrafish (Lu *et al*. 2017).

The maize *licc1* gene (GRMZM2G098797 gene; https://www.maizegdb.org/) encodes a homologue of the yeast *gid8* gene. We isolated a cDNA clone encoding *licc1* from a library constructed with mRNA from immature embryos 10 d after pollination (GenBank acc. No. KY348785). The gene *licc1* encodes a 226 amino acid protein with a predicted molecular mass of 25.8 kDa, which contains three conserved domains: LisH (Lissencephaly type-1-like homology), CTLH (C-terminal to LisH), and CRA (CT11-RanBPM) (Fig. 1*A*). We designate this protein as LICC1 (LisH-CTLH-CRA 1).

LICC1 shows similarity to proteins encoded by two other genes in the maize genome (GRMZM2G096877 and GRMZM2G085602), and to other plant, animal, and fungal proteins, including TWA1 and MAEA from humans and GID8 from yeast, components of the GID/MRCTLH protein complex. All these proteins contain the same three conserved domains (LisH, CTLH, and CRA).

A phylogenetic analysis was performed using the part of the proteins that encode the LisH, CTLH, and CRA domains (Fig. 1*B*). The maximum likelihood tree generated indicates that the sequences are divided into two main groups. Group 1 contains proteins of approximately 230 amino acids. It includes the human TWA1, yeast GID8, and the three maize proteins. Group 2 contains proteins of approximately 410 amino acids as the human MAEA. The differences in length between the groups 1 and 2 are mainly due to differences in the N- and C-terminal regions (with respect to the LisH, CTLH, and CRA domains). Group 1 can be further divided into two subgroups (1a and 1b). Subgroup 1b contains only sequences from *Viridiplantae* species with the sole exception of *Acanthamoeba castellanii.* Despite being part of the *Amoebozoa*, the *Acanthamoeba castellanii* genome contains a large number of plant genes as a consequence of extensive horizontal gene transfer from a plant or green algae (Clarke *et al*. 2013).

The association of several components of the human MRCTLH complex and the yeast GID complex with microtubules has been demonstrated (Salemi *et al*. 2017). Consequently, we decided to test whether the LICC1 protein from maize also interacts with microtubules. We used a co-sedimentation assay with LICC1 protein expressed in bacteria and taxol-stabilized microtubules assembled from purified tubulin (Fig. 2*A*). As a control, we

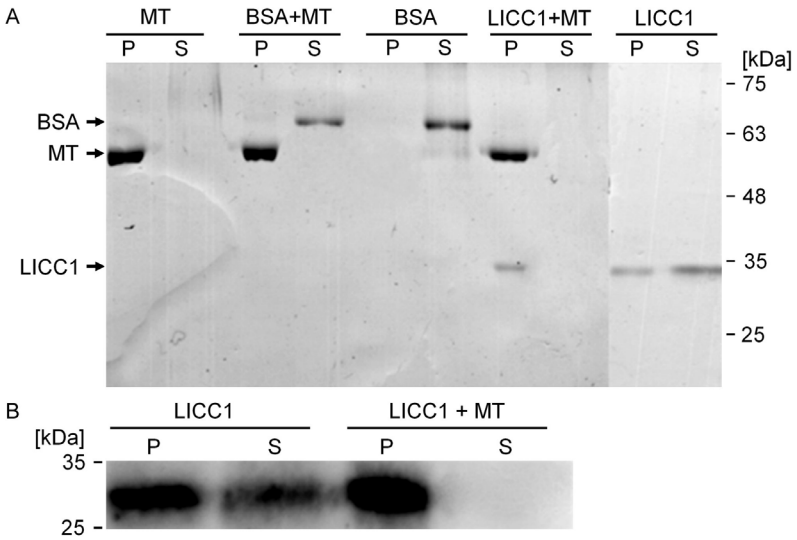

Fig. 2. LICC1 protein binds to microtubules (MT). *A* - Coomassie stained SDS-PAGE showing purified BSA (68 kDa) and tubulin (55 kDa), and bacterial expressed LICC1 protein (32 kDa). S are the soluble protein fractions and P are the precipitated protein fractions. MT, BSA, and MT+BSA are controls. LICC1 and LICC1+MTs correspond to the incubation of LICC1 in the absence or presence of microtubules, respectively. The full-length coding region of *licc1* (GenBank acc. No. KY348785) was cloned into pET28a and introduced in *Escherichia coli* BL21 cells. The MT-binding assays were performed using the *Microtubule Associated Protein Spin-Down Biochem Assay* kit (*Cytoskeleton Inc.*, Denver, USA) according to the manufacturer's recommendations. *B* - Immunoblots showing the purified LICC1 protein alone and mixed with microtubules (LICC1+MT). The anti-LICC1 antibody was generated against the peptide MASSKKVVTRDEWEC in rabbits (*Abyntek Biopharma SL*, Bizkaia, Spain).

used stabilized microtubules in the precipitated fraction and the soluble protein bovine serum albumin (BSA) in the soluble fraction (Fig. 2*A*). As controls, stabilized microtubules appeared in the precipitated fraction, the soluble protein BSA in the soluble fraction and BSA incubated in the presence of microtubules remains soluble. LICC1 is only partially soluble and appears in both soluble and precipitated fractions. However, when incubated with microtubules, all of the LICC1 protein appeared in the precipitated fraction, which suggests an interaction of LICC1 with microtubules.

To confirm these results, we transferred the SDS-PAGE to a nitrocellulose membrane which was then immunologically stained using an anti-LICC1 antibody (Fig. 2*B*). LICC1 alone is present in the soluble and in the precipitated fractions. LICC1 mixed with microtubules is only present in the precipitated fraction. These results indicate that LICC1 expressed in bacteria interacts with the microtubules.

Plant genomes contain multiple copies of the genes that encode the components of the MRCTLH/GID complex (Francis *et al*. 2013, Miquel and Vicient 2017). For example, there are three genes homologous to *twa1* and *gid8* in the maize genome. A larger number of genes can produce new variants of the protein complexes that can contribute to the development of new functions. The MRCTLH/GID complexes contain at least six proteins in plants. If each of them can appear in more than one variant, the possible number of versions of the complex is multiplied. Experimental data based on yeast two-hybrid assays indicate that there are at least two different MRCTLH/GID complexes in *Arabidopsis thaliana* and each appears to interact with a different set of proteins (Miquel and Vicient 2017). Considering that the single human complex has been involved in various regulatory functions, the presumed presence of multiple complexes in plants would mean that in plants the MRCTLH/GID complexes might be involved in the regulation of numerous processes.

Microtubules play a crucial role in numerous cellular processes in Eukaryotes, including intracellular transport, chromosome segregation, or cell division. To perform these functions, microtubules undergo rearrangements that involve cycles of assembly, disassembly, fragmentation, and interaction with other proteins (Garvalov *et al*. 2006). Our results indicate that LICC1 interacts with microtubules in a similar way to human TWA1, which associates with α-tubulin (Salemi *et al*. 2017). The interaction with microtubules is one of the proposed functions of the LisH domain (Delto *et al*. 2015). Several proteins containing LisH domains interact with microtubules, such as human LIS1, RanBPM, katanin p60, or *Arabidopsis* TONNEAU (Reiner and Sapir 2013). The roles of the interaction of human MRCTLH with microtubules is unclear, but in yeast, overexpression of *GID8* accelerates the onset of DNA replication and cells lacking *gid8* face a delay in DNA replication (Pathak *et al*. 2004). On the other hand, RanBPM, a member of the MRCTLH complex, has been shown to interact with histone deacetylase HDAC6, a microtubule-associated deacetylase that promotes many cellular processes leading to cell transformation and tumor development (Salemi *et al*. 2017).

In conclusion, we have shown that plant genomes contain multiple genes encoding proteins homologous to human TWA1 and yeast GID8. Plant genomes also contain genes that encode homologues of other components of the MRCTLH/GID protein complexes. Therefore, we can conclude that the MRCTLH/GID complexes are also present in plants, probably in multiple versions. The fact that at least some of their components interact with microtubules suggests a regulatory role similar to that in humans. Their precise functional roles remain to be determined.

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