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 (Valliyaveettil 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 1000 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 Vicent 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 it colocalizes 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. 1A). 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. 1B). 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 *Amebozoa*, 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. 2A). As a control, we
used stabilized microtubes in the precipitated fraction and the soluble protein bovine serum albumin (BSA) in the soluble fraction (Fig. 2A). As controls, stabilized microtubes appeared in the precipitated fraction, the soluble protein BSA in the soluble fraction and BSA incubated in the presence of microtubes remains soluble. LICC1 is only partially soluble and appears in both soluble and precipitated fractions. However, when incubated with microtubes, all of the LICC1 protein appeared in the precipitated fraction, which suggests an interaction of LICC1 with microtubes.

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

Plant genomes contain multiple copies of the genes that encode the components of the MRCTLH/GID complex (Francis et al. 2013, Miquel and Vicent 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 Vicent 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 microtubes suggests a regulatory role similar to that in humans. Their precise functional roles remain to be determined.

References
Clarke, M., Lohan, A.J., Liu, B., Lagkouvardos, L., Roy, S., Zafar, N., Bertelli, C., Schilde, C., Kianianmomeni, A., Bürglin, T.R., Frech, C., Turcotte, B., Kopec, K.O., Synnott, J.M., Choo, C., Paponov, I., Finkler, A., Tan, C.S.H., Hutchins, A.P., Weinmeier, T., Ratei, T., Chu, J.S.C., Gimenez, G., Irimia, M., Rigden, D.J., Fitzpatrick, D.A., Lorenzo-Morales, J., Bateman, A., Chiu, C.-H., Tang, P., Hegemann, P., Fromm, H., Raout, D., Greub, G., Miranda-Saavedra, D., Chen, N., Nash, P., Ginger, M.L., Horn, M., Schaap, P., Caler, L., Loftus, B.J.: Genome of Acanthamoeba castellanii highlights extensive lateral gene transfer and early evolution of tyrosine kinase signaling. - Gen. Biol. 14: R11, 2013.

Delto, C.F., Heisler, F.F., Kuper, J., Sander, B., Kneussel, M., Schindelin, H: The LisH motif of muskelin is crucial for oligomerization and governs intracellular localization. - Structure 23: 364-373, 2015.

Enes, R.D., Ponting, C.P.: A new sequence motif linking lissencephaly, Treacher Collins and oral-facial-digital type 1 syndromes, microtubule dynamics and cell migration. - Human mol. Genet. 10: 2813-2820, 2001.

Francis, O., Baker, G.E., Race, P.R., Adams, J.C.: Studies of recombinant TWA1 reveal constitutive dimerization. - Bioscience Rep. 37: BSR20160401, 2017.

Francis, O., Han, F., Adams, J.C.: Molecular phylogeny of a RING E3 ubiquitin ligase, conserved in eukaryotic cells and dominated by homologous components, the muskelin/ RanBPM/CTLH complex. - PLoS ONE 8: e75217, 2013.

Garvalov, B.K., Zuber, B., Bouchet-Marquis, C., Kudryashev, M., Gruska, M., Beck, M., Leis, A., Frischknecht, F., Bradke, F., Baumeister, W., Dubochet, J., Kyrklaff, M.: Luminal particles within cellular microtubes. - J. Cell Biol. 174: 759-765, 2006.

Lu, Y., Xie, S., Zhang, W., Zhang, C., Gao, C., Sun, Q., Cai, Y., Xu, Z., Xiao, M., Xu, Y., Huang, X., Wu, X., Liu, W., Wang, F., Kang, Y., Zhou, T.: Twa1/Gid8 is a β-catenin nuclear retention factor in Wnt signaling and colorectal tumorigenesis. - Cell Res. 27: 1422-1440, 2017.

Maitland, M.E.R., Onea, G., Chiasson, C.A., Wang, X., Ma, J., Moor, S.E., Barber, K.R., Lajoie, G.A., Shaw, G.S., Schild-Poulier, C.: The mammalian CTLH complex is an E3 ubiquitin ligase that targets its subunit muskelin for degradation. - Sci. Rep. 9: 9864, 2019.

Menon, R.P., Gibson, T.J., Pastore, A.: The C terminus of fragile X mental retardation protein interacts with the multi-domain Ran-binding protein in the microtubule-organising centre. - J. mol. Biol. 343: 43-53, 2004.

Menssen, R., Schweigert, J., Schreiner, J., Kusevic, D., Reuther,
J., Braun, B., Wolf, D.H.: Exploring the topology of the Gid complex, the E3 ubiquitin ligase involved in catabolite-induced degradation of gluconeogenic enzymes. - J. biol. Chem. 287: 25602-25614, 2012.

Miquel, M., Vicent, C.M.: Integrative meta-analysis of protein interaction data identified multiple GID/MRCTLH protein complexes in plants. - Plant Omics 10: 169-175, 2017.

Pathak, R., Bogomolnaya, L.M., Guo, J., Polymenis, M.: Gid8p (Dcr1p) and Dcr2p function in a common pathway to promote START completion in Saccharomyces cerevisiae. - Eukaryotic Cell 3: 1627-1638, 2004.

Reiner, O., Sapir, T.: LIS1 functions in normal development and disease. - Curr. Opin. Neurobiol. 23: 951-956, 2013.

Sahni, J.M., Mainland, M.E.R., Yetlet, E.R., Schild-Poulter, C.: Inhibition of HDAC6 activity through interaction with RanBPM and its associated CTLH complex. - BMC Cancer 17: 460, 2017.

Soni, S., Bala, S., Gwynn, B., Sahr, K.E., Peters, L.L., Hanspal, M.: Absence of erythroblast macrophage protein (Emp) leads to failure of erythroblast nuclear extrusion. - J. biol. Chem. 281: 20181-20189, 2006.

Suresh, B., Ramakrishna, S., Kim, Y.S., Kim, S.M., Kim, M.S., Baek, K.H.: Stability and function of mammalian lethal giant larvae-1 oncoprotein are regulated by the scaffolding protein RanBPM. - J. biol. Chem. 285: 35340-35349, 2010.

Tomaštíková, E., Cenklková, V., Kohoutová, L., Petrovská, B., Váchová, L., Halada, P., Kočárová, G., Binarová, P.: Interactions of an Arabidopsis RanBPM homologue with LisH-CTLH domain proteins revealed high conservation of CTLH complexes in eukaryotes. - BMC Plant Biol. 12: 83, 2012.

Umeda, M., Nishitani, H., Nishimoto, T.: A novel nuclear protein, Twal, and Muskelin comprise a complex with RanBPM. - Gene 303: 47-54, 2003.

Valiyaveettil, M., Bentley, A.A., Gursahaney, P., Hussien, R., Chakravarti, R., Kureishy, N., Prag, S., Adams, J.C.: Novel role of the muskelin-RanBP9 complex as a nucleocytoplasmic mediator of cell morphology regulation. - J. Cell Biol. 182: 727-739, 2008.

Yoo, K.W., Thiruvarangan, M., Jeong, Y.M., Lee, M.S., Maddirevula, S., Rhee, M., Bae, Y.K., Kim, H.G., Kim, C.H.: Mind bomb-binding partner RanBP9 plays a contributory role in retinal development. - Mol. Cell 40: 271-279, 2017.