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Toward the understanding of the role of CDC48, a major component of the protein quality control, in plant immunity

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Abstract

The evolutionally conserved chaperone-like protein CDC48 (cell division cycle 48) is a major component of ubiquitin-dependent protein degradation pathways in animal and yeast and, more generally, of the protein quality control machinery. In plants, CDC48 plays essential regulatory functions in development and the possibly that it contributes to protein degradation through the ubiquitin-proteasome system (UPS) and the endoplasmic reticulum-associated protein degradation (ERAD) system has been reported. In this review we described recent findings highlighting a role for CDC48 in plant immunity. First data indicated that CDC48 is S-nitrosylated in plant cells undergoing an immune response, regulates the turnover of immune receptors and mediates the degradation of viral proteins. Furthermore its overexpression was associated to an exacerbated hypersensitive-like cell death. We also designed and reported here the first CDC48 interactome. The corresponding data confirms the closed interaction of CDC48 with components of the UPS and shed light on its putative regulatory function of S-adenosyl-methionine synthesis and metabolism. More generally, these investigations further support the concept that plant cells facing pathogen attack finely regulate the protein quality control machinery.

Highlights

- The chaperone-like protein CDC48 is a component of the ubiquitin-proteasome system
- CDC48 contributes to the turnover of immune receptors
- CDC48 is mobilized by cells facing an immune response
- Overproduction of CDC48 leads to exacerbated cell death during the immune response
- The *A. thaliana* CDC48 interactome highlights new functions of CDC48

Keywords

CDC48; Interactome; Plant immunity; S-adenosyl-methionine; Ubiquitin-proteasome system

1 - Introduction

The protein synthesis and degradation systems remain in balance in order to maintain cell functions. In animals and yeast, it is now well established that the proper flux of proteins is highly regulated by numerous factors that finely control the precise abundance of each protein as well as the stoichiometry of protein complexes. Dysregulation of this process has been linked to the development of multiple pathologies such as Alzheimer's disease [1]. Accordingly, cells facing increased translational input, including tumorigenic cells, elevate the protein quality control by enhancing their protein folding and degradation capacities, notably through the synthesis of heat shock proteins (HSPs) and proteasome subunits. This adaptation also reduces the risk of proteotoxic stress due to proteome imbalance [2, 3]. The protein quality control is therefore the focus of intense research in human therapies and, for instance, drugs targeting proteins of the protein balance machinery, such as HSP90 or proteasome subunits, are developed as strategies to kill tumor cells.

Plants undergoing an immune response show enhanced transcriptomic and translational activities. In addition to the protein biogenesis machinery, transcriptomic and proteomic studies highlighted that protein degradation is up-regulated in plant facing pathogen attack. In particular, genes encoding key players of the ubiquitin-proteasome system (UPS) such as E3-ubiquitin ligases and 26S proteasome subunits are over-expressed during plant immune responses [4-7], suggesting that the increased protein biosynthesis is accompanied by an enhanced proteolysis in order to maintain the protein balance. The UPS also regulates the accumulation of key players of the immune responses, including intracellular nucleotide-binding leucine-rich repeat (NB-LRR) immune receptors involved in the direct or indirect perception of effectors [4, 8]. Interestingly, the turnover of immune receptors and, therefore, proper defence responses, also involve chaperones as reported for HSP90 [9]. Completing this picture, chaperones contribute to plant defense through their interaction with effectors as shown for HSP70 [10]. In turn micro-organisms and viruses are able to manipulate components of the UPS by redirecting this proteolysis machinery to eliminate unwanted host cell proteins and/or to regulate their own protein homeostasis [11-14].

The conserved AAA+ ATPase CDC48, also named valosin-containing protein (VCP) or p97 in metazoans, is a major cytosolic and nuclear component of the protein quality control where it acts in ubiquitin-mediated pathways [15-17]. CDC48 consists of a C-terminal (C-ter) and N-

terminal (N-ter) flexible domains. The N-ter domain contributes to the binding of ubiquitinated client proteins and both the N- and C-ter domains interact with cofactors. The core of the protein contains two adjacent ATPase domains (namely, D1 and D2), both of which containing a Walker A and Walker B motif involved in the binding and hydrolysis of ATP, respectively [18, 19]. CDC48 is active as a stable homo-hexameric barrel structure with a small central pore [20]. It is now commonly accepted that the binding of ATP to the D1 domain promotes the hexamerization of the protein whereas the ATPase activity of the D2 domain is required for CDC48 biological function. Most of our knowledges regarding CDC48 activities come from studies based on animal and yeast models. This protein is associated with main cellular processes including the regulation of cell cycle, DNA replication and damage response, gene expression, membrane fusion, autophagy and apoptosis [15, 21]. At the molecular level, the main role of CDC48 resides in the extraction of poly-ubiquitinated client proteins from membranes, protein complexes and chromatin upon ATP hydrolysis by the D2 domain. Therefore, CDC48 acts as a segregase. Then, client proteins are subsequently targeted to the proteasome for degradation [16, 20, 22]. The extraction is based on the ATP-dependent unfolding of the client proteins through their transit in the central pore. This process is particularly energy consuming as the D2 domain hydrolyses ATP many times to pull the client proteins through the central pore. A deubiquitinase (DUB) assists CDC48 in order to reduce the length of the ubiquitin chain, thus facilitating the translocation of the protein through the pore [18, 23]. The role of CDC48 is best exemplified by its involvement in the endoplasmic reticulum (ER)-associated protein degradation (ERAD) and mitochondrion-associated degradation (MAD) pathways in which it interacts with and extracts poly-ubiquitinated misfolded proteins from the ER and mitochondria before deliver them to the proteasome for proteolysis [15, 24, 25]. In addition, CDC48 acts in the ribosome-associated degradation (RAD) pathway to remove the defective nascent polypeptide chains resulting from errors in translation before their degradation [16, 26]. Finally, regarding immunity, a dual role for CDC48 during viral infection has been reported in humans. In one hand, CDC48 emerges as an important host factor in antiviral immunity, for instance by contributing to the intracellular neutralization of adenovirus mediated by the cytosolic antibody receptor TRIM21 [27]. On the other hand, CDC48 is required for cytomegalovirus replication by favouring the expression of the virus gene IE2 [28] as well as for the replication of the West Nile virus [29].

Homologs of CDC48 were identified in plants. For instance, according to BLAST search the genome of *Arabidopsis thaliana* contains 5 CDC48 paralogs. *AT3G09840*, *AT3G53230*, *AT5G03340* encoding AtCDC48A, AtCDC48B and AtCDC48C, respectively, share a high degree of sequence similarity with mammalian and yeast *CDC48*. The two other isoforms, namely AtCDC48D and AtCDC48E encoded by *AT2G03670* and *AT3G01610*, respectively, show more distantly related sequences [30]. Not much is known about the function of CDC48 in plants. It was first reported that in *A. thaliana*, CDC48 contributes to cell division, cytokinesis and growth processes and was preferentially expressed in expanding cells in contrast to morphologically differentiated cells [31, 32] . Accordingly, T-DNA mutants impaired in the expression of the *A. thaliana* AtCDC48A isoform showed embryo growth arrest at the early stage of development and seedling lethality [33]. The AtCDC48E paralog also appeared to be required for normal growth and development [30]. Similarly, mutation or invalidation of the expression of CDC48 in rice led to a premature senescence and death phenotype [34]. In contrast, T-DNA knockout alleles of the B, C and D paralogs of *A. thaliana* CDC48 were viable and displayed normal morphology, suggesting that the *A. thaliana* CDC48 paralogs might have different functions (see also subchapter 2.1) [30]. A role for plant CDC48 in UPS and ERAD were also evidenced in several investigation. For instance, when over-expressed in tobacco protoplasts in the absence of its partner B subunit, the catalytic A subunit of the castor bean toxin ricin was shown to be retro-translocated from the ER lumen to the cytosol and degraded through a CDC48-dependent process [35]. According to the author, this mechanism allows the elimination of orphan proteins that fail to assemble correctly. Supplementing this data, Yamamoto *et al*. [36] demonstrated that upon its expression in *A. thaliana* culture cells, a vacuolar carboxypeptidase mutated in its active site was retained in the ER and next degraded by the proteasome in a CDC48-dependent manner. This finding illustrates a role for CDC48 in removing non-functional proteins. Other client proteins of CDC48 were identified such as the soluble secretory protein CLAVATA3 [37], the small GTPase ARF1 involved in subcellular trafficking [38], immune receptors [30] and proteins involved in various cellular processes (see the following sub-chapters for further details).

In terms of regulation, AtCDC48A appears to be primarily hexameric in living cells [39] and regulated by AtPUX1, an ubiquitin-regulatory X (UBX) domain-containing protein which inhibits AtCDC48A activity and promotes the disassembly of the active hexamer [40, 41]. Two other UBX domain-containing proteins, AtPUX7 and AtPUX10, were reported to interact

with AtCDC48A. Concerning AtPUX7, the interaction occurs in the nucleus and could constitute a molecular adaptor between AtCDC48A and ubiquitinated substrates [42]. AtPUX10 was shown to be localized to lipid droplets (LD) in pollen tubes and seeds and to recruit AtCDC48A [43, 44]. Authors of these recent investigations proposed that this interaction is part of a degradation process of ubiquitinated LD proteins including oleosins which play a key function in LD stability. Finally, AtCDC48A was shown to interact at the plasma membrane and to be phosphorylated by the somatic embryogenesis receptor-like kinase 1 receptor (SERK1) [39, 45]. A redox-based regulation through nitric oxide (NO)-induced post-translational protein modification has also been demonstrated [46].

Here, we describe and discuss recent findings highlighting a role for CDC48 in plant immunity and generated a CDC48 protein-protein interaction network providing a detailed overview of CDC48 involvement in defense and, more generally, of its functions in plant cells.

2.1 - CDC48 involvement in plant immunity

An increasing body of evidence suggests that CDC48 plays an important role in immunity as a component of the UPS machinery. First, in *A. thaliana* plantlets infected by the oilsseed rape mosaic virus (ORMV), the AtCDC48A isoform was shown to mediate the retro-translocation of ORMV movement proteins (MPs) from the ER toward the cytosol for their subsequent degradation [47]. The purpose of this process is not clearly understood as, according to the authors, it might contribute to either plant defense or to the regulation of ORMV replication [48]. These opposite hypotheses highlight two scenarios previously proposed by Verchot [14]. In one hand, viral interactions with the UPS components such as CDC48 but also ubiquitin ligases could serve to regulate virus infection in order to facilitate rapid turnover of viral proteins and to promote replication and movement. In the other hand, the UPS machinery could participate to antiviral immunity by degrading viral effectors. A role for AtCDC48A in the turnover of the NB-LRR protein SNC1 *via* its degradation through the 26S proteasome was also proposed in plantlets facing *Hyaloperonospora arabidopsidis* Noco2 infection [30]. This mechanism is central in the regulation of the NLR protein abundance as an increase in SNC1 level leads to autoimmunity. Importantly, this role was specifically attributed to AtCDC48A and not to the other *A. thaliana* paralogs, raising again the possibility that the AtCDC48 paralogs have diversified functions. Further supporting a role

for the UPS machinery in the regulation of SNC1 levels, down-expression of the ubiquitin E3 ligase CPR1 (Constitutive expressor of Pathogenesis Related genes 1) or of the E4 protein MUSE3 (Mutant, SNC1-Enhancing 3) that further poly-ubiquitylates SNC1, led to enhanced NLR accumulation and autoimmunity [49, 50]. Completing these data, in response to the tomato yellow leaf curl virus an increase of the amount of CDC48 protein was observed in the susceptible tomato cultivar *Jinpeng-1* as compared to the resistant cultivar *Zheza-301* [51]. The authors did not investigate the role of CDC48 in this patho-system.

Further arguments highlighting a function of CDC48 in plant immunity were recently reported thanks to the cryptogein/tobacco model. Cryptogein is a 10 kDa elicitin produced by *Phytophthora cryptogea*. This oomycete, or purified cryptogein, triggers a hypersensitive response (HR) as well as a systemic acquired resistance (SAR) in tobacco plants [52]. The mechanisms underlying cryptogein-induced immune responses have been widely studied in tobacco cell suspensions [53, 54]. In these latter, both NtCDC48 protein and transcripts were shown to accumulate in response to cryptogein treatment [38]. The protein accumulated under its hexameric stable structure, suggesting that its activity was increased during the immune response. Accordingly, the cell death induced by cryptogein appeared to be accelerated and more pronounced in a tobacco cell lines overexpressing NtCDC48. These cells did not show an increased level of reactive oxygen species (ROS) and NO, two main components of the cryptogein-induced cell death pathway [54]. Therefore, NtCDC48 overexpression might contribute to cell death through a ROS/NO independent process.

Interestingly, CDC48 was also found to undergo a fast S-nitrosylation in tobacco cells facing cryptogein exposure [46]. S-nitrosylation is a reversible NO-dependent post-translational protein modification of cysteine residues (Cys) thiol leading to nitrosothiol (SNO) [55]. Cys526 was identified as a target for S-nitrosylation *in vivo* whereas *in vitro* two other Cys residues were shown to be S-nitrosylated in addition to Cys526. This latter is located in the Walker A motif of the D2 domain involved in the binding of ATP. *In vitro*, its S-nitrosylation was shown to suppress NtCDC48 ATPase activity and to trigger a slight conformational change of the protein. According to the authors, the S-nitrosylation of Cys526 could interfere with ATP binding to the protein through a mechanism of steric hindrance. Other proteomic studies aiming at identifying S-nitrosylated proteins in various plant species under distinct physiological conditions also led to the identification of CDC48 (for review see [55]). In particular, the protein was shown to be prone to S-nitrosylation in the nucleus of *A. thaliana*

cells infected with *Pseudomonas syringae* [56]. Therefore, S-nitrosylation emerges as an important post-translational regulation of CDC48. This observation raises the question of the impact of this process *in vivo*. In this regard, Rosnoblet *et al*. [38] demonstrated that in response to cryptogein treatment only a few percent of the cellular NtCDC48 population undergoes S-nitrosylation, reinforcing the difficulty in understanding the physiological incidence of this post-translational modification. The hypothesis that CDC48 S-nitrosylation constitutes a redox-based signal activating the UPS machinery has been proposed but not supported by experimental evidences [57].

In animals and yeast, partners of CDC48 vary according to the cellular process in which it is involved. In plants, CDC48 has been reported to form complexes with defense-related proteins such as SERK1 in rice, providing insights into its mode of action in immunity [45, 58, 59]. Based on these statements, a proteomic approach aiming at identifying NtCDC48 partners in cryptogein-treated tobacco cells has been recently carried out through endogenous proteins immunoprecipitation followed by mass spectrometry analysis (IP-MS) [38]. The corresponding peptides identified by MS were BLAST against the SwissProt/Trembl *Viridiplantea* database and only proteins with more than 90 % of identity with *Nicotiana tabacum* proteins were considered as partners of CDC48. The majority of NtCDC48 partners identified was related to primary metabolism and cellular energy. Some functional classes were particularly relevant of its function well known in mammals and yeast, notably those related to protein quality control and UPS, gene expression and subcellular trafficking. Accordingly, several proteins involved in these pathways have already been identified as CDC48 partners in animals and yeast, such as the ADP ribosylation factor ARF, polyubiquitin, proteasomal subunits proteins or 14.3.3 members. Specific features of the plant CDC48 proteome also emerged from this analysis and are further discussed below. Interestingly, the MS analysis highlighted that many identified peptides were oxidized, underlining again the chaperone-like function of CDC48. Of importance, although these experiments led to the identification of a hundred of partners, none of them were found exclusively in cryptogein-treated cells.

2.2 - CDC48 Interactome

2.2.1 - Establishment of the CDC48 interactome

To further investigate the role of CDC48 in plant immunity, its protein interaction network was constructed based on the NtCDC48 interactome previously characterized by Rosnoblet *et al*. [38] .

The protein-protein interaction databases are poorly enriched in tobacco proteins or in proteins from other Solanaceae. In order to benefit from *A. thaliana* databases, we developed the strategy sum up Figure 1. First, each tobacco putative protein (n = 4705) from the previous MS analysis [38] were blasted against TAIR database containing the whole *A. thaliana* proteome (version: 2018-01-05). This step allowed a more exhaustive identification of proteins interacting with CDC48 as only 125 proteins (3 percent of the total pool of peptides) remained unidentified versus 1825 proteins in the initial analysis in tobacco. Next, because of the change of species, we applied to the 4580 remaining proteins a first sorting in order to keep the tobacco proteins showing at least 70% of identity with their *A. thaliana* counterparts. Consequently, 3898 proteins were excluded. Then, the 682 remaining proteins were subjected to a second sorting in which we selected the proteins found in a reproducible way in the five independent IP-MS experiments performed by Rosnoblet *et al*. [38]. At the end, 128 putative *A. thaliana* CDC48 partners were identified versus 97 in the previous analysis restricted to tobacco. Amongst these, 126 were considered as recurrent partners of CDC48 as they co-immunoprecipitated in both cryptogein and control conditions. The two other proteins were found only in the protein list corresponding to cryptogein treatment and, consequently, emerged as CDC48-specific partners in cells undergoing an immune response. One of these two proteins, AtEBP1 (or AtG2) encoded by *AT3G51800*, is homologue of the human epidermal growth factor receptor EBP1 and was shown to regulate organ size and cell proliferation [60, 61]. The second one is an Arginine-tRNA ligase. Mutation of the corresponding gene, *AT4G26300*, was described as embryo-lethal [62].

A two way reciprocal BLASTp search was conducted between the *A. thaliana* proteins found in this study and the *N. tabaccum* CDC48-interacting proteins identified in Rosnoblet *et al*. [38]. This reciprocal BLASTp comparison confirmed that a large percentage of *N. tabacum* partners found an *A. thaliana* orthologue (Table 1). Actually, 85 of a total of 97 *N. tabaccum* proteins found mutual best hits with a stringent e-value of 10^{-10}, or better. Interestingly, only 64 orthologs were identified, meaning that some tobacco partners reciprocal BLASTp led to the same *A. thaliana* ortholog. Thus, amongst the 128 putative *A. thaliana* CDC48 partners (Figure 1), 31 could be considered as newly identified partners of CDC48, including EBP1 and

the Arginine-tRNA ligase. Among those proteins, many isoforms of previously identified partners were found. For instance, number of isoforms of tubulin, S-adenosyl-methionine (SAM) synthase, UDP-glucose-6-dehydrogenase, glyceraldehyde 3 phosphate dehydrogenase, 14-3-3 proteins, actin, or fructose-bisphosphate aldolase were identified. This observation suggests that CDC48 may not be specific to one isoform but can interact with several isoforms of a same protein family. Moreover, among the newly identified proteins, an enrichment of members of protein complexes occurred. For instance, in the *N. tabaccum* CDC48 interactome, only two subunits of the regulatory particle of the 26S proteasome were identified. Here, 5 subunits were found, including the non-ATPase regulatory subunits RPN1A, RPN7 and RPN3A and the ATPase regulatory subunits RPT4B and RPT3. The same tendency was observed for isocitrate dehydrogenase and ATP synthase complexes. These results indicate that the plant CDC48 not only interacts with single proteins but with protein complexes as previously reported in other organisms [17, 63].

2.2.2 - Analysis CDC48 partners

Next, a deeper characterization of the CDC48 interactome was performed. Although protein functions can be described in multiple ways, we focused on the classification schemes provided by the Gene Ontology (GO) Consortium [64]. For this purpose, we used the Protein Analysis Through Evolutionary Relationship (PANTHER) database [65]. PANTHER (http://www.pantherdb.org/) was designed to classify proteins in order to facilitate high-throughput investigations. Thus, our total protein set was analyzed according to the protein classes, molecular functions, biological processes or pathways. Based on this parameters, the protein partners have been classified into 13 different protein classes (Figure 2A), confirming that CDC48 interacts with a wide range of different proteins and is potentially involved in various cellular activities. Main classes include hydrolases and oxidoreductases. Accordingly, the largest part of the molecular function GO term assignation (Figure 2B) and of the biological process GO term (Figure 2C) were catalytic activity and metabolism. Proteins related to the nucleic acid regulations, to cytoskeleton, membrane traffic and protein folding also emerged from this analysis.

We further exploited our data using statistical overrepresentation test of the PANTHER database. This tool is based on the simple binomial test and compares a protein list to a reference list and determines whether a particular class of proteins is overrepresented or

underrepresented [65]. Therefore, we compared the list of the protein partners of CDC48 to the whole proteome of *A. thaliana*. The analysis of the GO Term indicated that CDC48 partners involved in 4 biological pathways are overrepresented (Figure 2D). These pathways include the ubiquitin proteasome pathway. This data was expected as CDC48 is a major component of the UPS [63] and confirms the validity of our approach. Proteins involved in energy supply, notably glycolysis and synthesis of ATP were also found enriched, further supporting the observation that CDC48 particularly interacts with proteins related to metabolism. Interestingly, proteins involved in the biosynthesis of SAM were strongly overrepresented. SAM is a molecule located at the crossroad of amino-acids and ethylene biosynthesis and is involved in iron homeostasis through the production of nicotianamine [66, 67]. By acting as a methyl group donor, SAM also acts as cofactor in number of metabolic reactions and contributes to the regulation of protein activities through post-translational modifications.

2.2.3 - Comparison with human and yeast CDC48 interactomes

We decided to highlight the specificity of the plant CDC48 interactome as compared to other organisms. For this purpose, we compared the plant CDC48 interactome with those of human (VCP) and yeast (ScCDC48) CDC48. In order to properly compare these interactomes, we extracted from the Biogrid database (https://thebiogrid.org/; version: 3.4.156) partners of VCP and ScCDC48 that were identified by affinity capture followed by MS analysis. We found 446 and 104 VCP and ScCDC48 partners, respectively. Then we performed the same GO Term analysis as described above.

According to the protein classification, VCP partners belong to 22 protein classes versus 11 for the ScCDC48 partners (Figure S1) and 13 for the plant partners (Figure 2A). The higher number of protein classes in the human interactome might be linked to the higher number of VCP partners identified so far. Nine classes of CDC48 interactants were similarly found in all organisms (Figure 3A). Proteins annotated nucleic acid binding and hydrolases were predominant, notably in yeast (Figure 3B). CDC48 also commonly interacts with chaperones, ligases, transferases and with proteins related to membrane traffic. Oxidoreductases and transporters were found both in human and plants but not in yeast. Similarly, cytoskeletal proteins were less represented in the yeast CDC48 interactome. Isomerase was the unique protein class found exclusively in the plant CDC48 interactome but transcription factors were

less represented (Figure 3B). More generally, although CDC48 activities have been linked to defense responses, this survey clearly indicates that CDC48 does not specifically interact, or poorly, with defense/immunity proteins (Figure S1).

According to the molecular function and biological process analyses, again CDC48 partners were mainly involved in catalytic activities and metabolism (Figure 2B and C), whatever the organism (Figure S2). In contrast, signal transducers activity appeared to be restricted to the human VCP partners whereas anti-oxidant activities were more abundant in the plant CDC48 partners list. Indeed, we previously identified cytosolic ascorbate peroxidase, catalase and superoxyde dismutase, three main redox-related proteins, as CDC48 partners in tobacco cells [38].

Thanks to the PANTHER overrepresentation tool, we finally highlighted that SAM biosynthetic process, glycolytic process and pentose-phosphate shunt were GO terms specifically overrepresented in the plant CDC48 interactome, as compared to the human and yeast interactome (Figure 3C). Some biological processes were also found enriched in both the human and plant interactomes, as the regulation of mitochondrial membrane permeability, the response to the hydrogen peroxide and protein folding (Figure 3D). At the opposite some well-described biological processes involving CDC48 as autophagy, ubiquitin-dependent ERAD pathway or chromatin remodeling were found enriched only for the Human and Yeast interactome (Figure 3E).

2.2.4 - Analysis of the CDC48 protein-protein interaction (PPI) network

Thanks to the new list of CDC48 partners, we constructed the protein-protein interaction network representing only CDC48, its partners and their respective interactions. For this purpose, the BioGRID database in which experimental interactions are reported was used [68]. Only physical interactions were taken into account and no distinctions were made concerning AtCDC48 isoforms. The resulting network was composed of 129 proteins, symbolized by nodes, and 236 interactions symbolized by links (Figure 4A). Based on this network, specific groups of CDC48 partners were designed. More precisely, protein communities, highlighting groups of interconnected proteins, were defined. Communities are network modules which are densely connected within themselves but sparsely connected with the rest of the network. They generally correspond to meaningful biological units such as protein complexes and functional modules [69]. For communities detection,

several algorithms could be used but we choose the Louvain one [70] which gives one of the best coefficient of modularity and was one of the best to discriminate protein complexes.

Thus, 7 communities were generated (Figure 4B). Among them, 2 major communities with a large number of proteins were obtained. The first one is composed by CDC48 and proteins that, for the majority, interact only with it. The two protein partners interacting with CDC48 only during elicitation, EBP1 and Arginine-tRNA ligase, were identified as belonging to this community. The second larger community is composed essentially by proteins that interact both with CDC48 and the ubiquitin UBQ3 (encoded by *AT5G03240*). All the other communities interacted with largest communities. As previously mentioned, with this method we were able to discriminate proteins described as membership of protein complexes. Notably, 5 proteins composing the 26S proteasome were grouped together (major node RPN1A encoded by *AT2G20580*) as well as the isocitrate dehydrogenase subunits (major node IDH1 encoded by *AT4G35260*). Two others communities, with intermediate sizes, were composed by proteins interacting with central proteins belonging to the 14-3-3s family (major nodes GRF2 and GRF8 encoded respectively by *AT1G78300* and *AT5G65430*). These two communities were also found to interact together. The last community was composed by three chaperones proteins (major node CR88 encoded by *AT2G04030*).

As the algorithm chosen to define communities was able to detect protein complexes, we investigated whether communities could be described as functional modules. Thus, the presence of biological pathways within communities was analyzed. We used the Kyoto Encyclopedia of Genes and Genomes (KEGG; https://www.genome.jp/kegg/) database to recover information about pathways in which CDC48 and its partners were involved. These information were attributed to 95 proteins over 129. Such analysis makes sense if the number of proteins into a community is large enough. This explains why we performed it only for communities represented by CDC48, UBQ3 and both 14-3-3 members (GRF2 and GRF8).

For the communities of proteins interacting only with CDC48 or both CDC48 and UBQ3, a large number of pathways were found without any over-representation of one or several pathways. For communities represented by 14-3-3 members, especially for GRF8, an important part of the pathways pool was composed by proteins involved in cysteine and methionine biosynthesis (KEGG ID: ath00270; https://www.genome.jp/kegg-

bin/show_pathway?ath00270). Herein, the four proteins involved into this pathway correspond to the four isoforms of SAM synthase (*AT3G17390*, *AT4G01850*, *AT2G36880* and A*T1G02500*) that catalyse the formation of SAM from methionine and ATP. The last protein involved in this pathway and belonging to this community is the cytosolic malate dehydrogenase 1 (MDH1; *AT1G04410*) which is at the interface of this amino acid biosynthesis and pyruvate metabolism. Concerning the cysteine and methionine biosynthesis pathway, the cysteine synthase 1 (OSA1; *AT4G14880*) was also found into the community represented by GRF2. OSA1 catalyses the synthesis of cysteine from O-acetyl-L-serine and hydrogen sulphide.

Two other proteins involved in the cysteine and methionine biosynthesis were found into the community represented by UBQ3. Interestingly, these enzymes are closely link to SAM biosynthesis (Figure 4C). The first enzyme is homocysteine methyltransferase (MS1; *AT5G17920*) that catalyzes the transfer of a methyl group to homocysteine resulting in methionine, one of the precursor of SAM. The second enzyme, adenosyl-homocysteinase (SAHH1, *AT4G13940*) hydrolyses S-adenosyl homocysteine into L-homocysteine. S-adenosyl homocysteine is the molecule formed after the transfer of the methyl group from SAM to an acceptor.

3 - Discussion

With the identification of CDC48 protein partners and the establishment of its protein network, we are now gaining insight into its function. The data highlighted here provide evidence that CDC48 interacts with components of the immune response as exemplified with SNC1 and SERK1. These processes contribute to the proper turnover of SNC1 and CDC48 post-translational regulation, respectively. Other proteins such as AtEBP1 and arginine-tRNA ligase might constitute targets of CDC48 during defense responses, although we lack experimental evidences supporting this hypothesis. As reported in animals, CDC48 also interacts with and promotes the degradation of viral proteins as shown for the ORMV MPs. The question of whether this process constitutes a viral strategy or a defense mechanism remains unanswered.

The analysis of CDC48 involvement in cryptogein-induced immune response and the deduced CDC48 protein network are informative on several fronts:

- First, the CDC48 interactome advances our understanding of its cellular functions. Undoubtedly, the identification of ubiquitin, of subunits of the 26S proteasome as well as chaperones as partners confirms its central role in the protein quality control and notably in UPS. Unexpectedly, the protein-protein network also reveals a close connection of CDC48 with SAM biosynthesis and metabolism. As previously mentioned, SAM is an abundant plant co-factor considered as an activated form of methionine. It is a main precursor of the ethylene, nicotianamine and polyamine biosynthetic pathways which are mobilized in numerous physiological processes including plant defense. The role of CDC48 in the regulation of SAM-dependent metabolic pathways is currently unknown but deserves particular attention. More generally, a majority of CDC48 partners are related to primary metabolism, an enrichment of proteins associated to the glycolysis and ATP synthesis being observed. It is likely that this data reflects a high turnover of the corresponding proteins in order to meet the cellular energy requirements.

- Second, with the exception of AtEBP1 and arginine-tRNA ligase, the CDC48 partners did not differ among the cryptogein-treated and control cells. This conclusion must be nuanced as the identification of the CDC48 partners developed by Rosnoblet *et al*. [38] was qualitative but not quantitative. Nevertheless, as cryptogein was shown to trigger an increased accumulation of NtCDC48 in its hexameric active structure, it is plausible to assume that the segregase activity of NtCDC48 and, more generally, the UPS machinery are over-induced in cells undergoing an immune response. Accordingly, in addition to NtCDC48, transcripts encoding E3-ligases, 26S proteasome subunits as well as HSPs were shown to accumulate in cryptogein-elicited cells [54, 71, 72]. As the transcriptional activity of these latter is up-regulated, the over-accumulation of CDC48, of components of UPS and of the folding machinery probably reflects a process allowing cells to face the increased protein biogenesis and inherent risks of imbalance in the proteome caused, for instance, by errors in translation, accumulation of misfolded proteins or orphan protein subunits. Furthermore, UPS might contribute to the degradation of oxidized proteins caused by ROS.

- Third, transgenic cells over-expressing NtCDC48 showed a faster and amplified cryptogein-induced cell death. The underlying mechanisms have not been investigated so far. One possibility would be that CDC48 interacts with and promotes the degradation of negative regulators of cell death such as histone deacetylases of type II [53]. However, such regulators were not identified in the list of CDC48 partners. The fact that nuclear proteins

were excluded from samples during protein extraction could partly explain this statement. Another possibility deserving attention is that in cells over-expressing NtCDC48, the induced accumulation of the endogenous CDC48 triggered by cryptogein, together with the accumulation of the product of the transgene, could lead to an exacerbated concentration of active CDC48 and, consequently, to higher segregase activities. In this scenario, a proteome imbalance in favour of protein degradation might occur, thus favouring cell death.

A future challenge is to understand the impact of the interaction between CDC48 and partners of interest. Indeed, in addition to trap proteins and deliver them to the proteasome to assume proper protein turnover, such interactions might also be part of other cellular processes that remain to be characterized. Another promising aspect is to extend the analysis of the CDC48 protein network to other patho-systems, including plant-virus interactions. Such approach could also lead to the identification of microbial proteins degraded through or manipulating CDC48 during infections. Finally, a further challenge is to complete the qualitative identification of CDC48 partners with quantitative analysis.

4 - Acknowledgment

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678
679 **Figure legends**
680
681
682 Figure 1 - Schematic representation of the procedure used for generating the CDC48
683 interactome. In the initial IP-MS analysis [38], 5 independent experiments, containing each
684 control and cryptogein-treated samples, were performed. See the text for details.
685
686 Figure 2 - Description of the CDC48 interactome *via* Gene Ontology analysis
687 A,B and C) Pie charts representing the protein classes, molecular functions and biological
688 processes related to the plant CDC48 partners, respectively. The analysis was performed
689 thanks to the PANTHER database classification tool.
690 D) Biological pathways overrepresented in the CDC48 interactome. This analysis was
691 performed thanks to the PANTHER database overrepresentation tool. The
692 overrepresentation is statistically inferred using the Fisher's Exact with False Discovery Rate
693 (FDR) multiple test correction. Results display p-values < 0.05 [65].
694
695 Figure 3 - Comparison of the plant CDC48 interactome to VCP and Yeast CDC48 interactomes
696 *via* Gene Ontology analysis
697 A) UpSet representation of protein class intersections between Plant, Human and Yeast
698 CDC48 interactomes. The bar plots indicate the number of protein classes found in each
699 model organism; columns correspond to the number of protein classes found in one, two or
700 in the three model organisms (exact number is specified above each column). Below a given
701 column, the black points indicate in which organism(s) those protein classes are found. If
702 only one point is represented, this indicates that the protein classes composing the given set
703 are specific to one organism. For instance, 8 protein classes are specific to the human CDC48
704 interactome and are not found in yeast and plants. In contrast, 9 protein classes are similarly
705 found in the CDC48 interactomes of human, yeast and plants. Protein classes were extracted
706 from the PANTHER database classification tool.
707 B) Proportion (in %) of the 13 protein classes composing the plant CDC48 interactome
708 (green) and, when possible, the proportion of the same protein classes into the human (red)
709 and yeast (blue) CDC48 interactomes. For a given protein class, star indicates the proportion

that are statistically different between organisms using pairwise proportion test (p-value < 0.05). Protein classes were extracted from the PANTHER database classification tool.

C) Biological processes (BP) GO terms that are found overrepresented in the plant interactome.

D) Common BP GO terms found to be overrepresented in human and plants CDC48 interactomes.

E) Common BP GO terms found to be overrepresented in yeast and human CDC48 interactomes.

For figures C, D and E, BP GO Term analysis was performed thanks to the PANTHER database overrepresentation tool. The overrepresentation of a given BP GO Term in a given organism is statistically inferred using the Fisher's Exact with FDR multiple test correction. Results display p-values < 0.05.

Figure 4 - Protein-protein interaction (PPI) interaction network of the plant CDC48 interactome

A) Representation of the PPI network of CDC48. Nodes represent proteins and the size of nodes is proportional to the number of connections with other nodes as known as degree. Links between nodes represent physical interactions between proteins. These links were either highlighted in Rosnoblet *et al*. study [38] or from the Biogrid database. Each color represents nodes belonging to a same community. The graphic representation was performed thanks to the Gephi software.

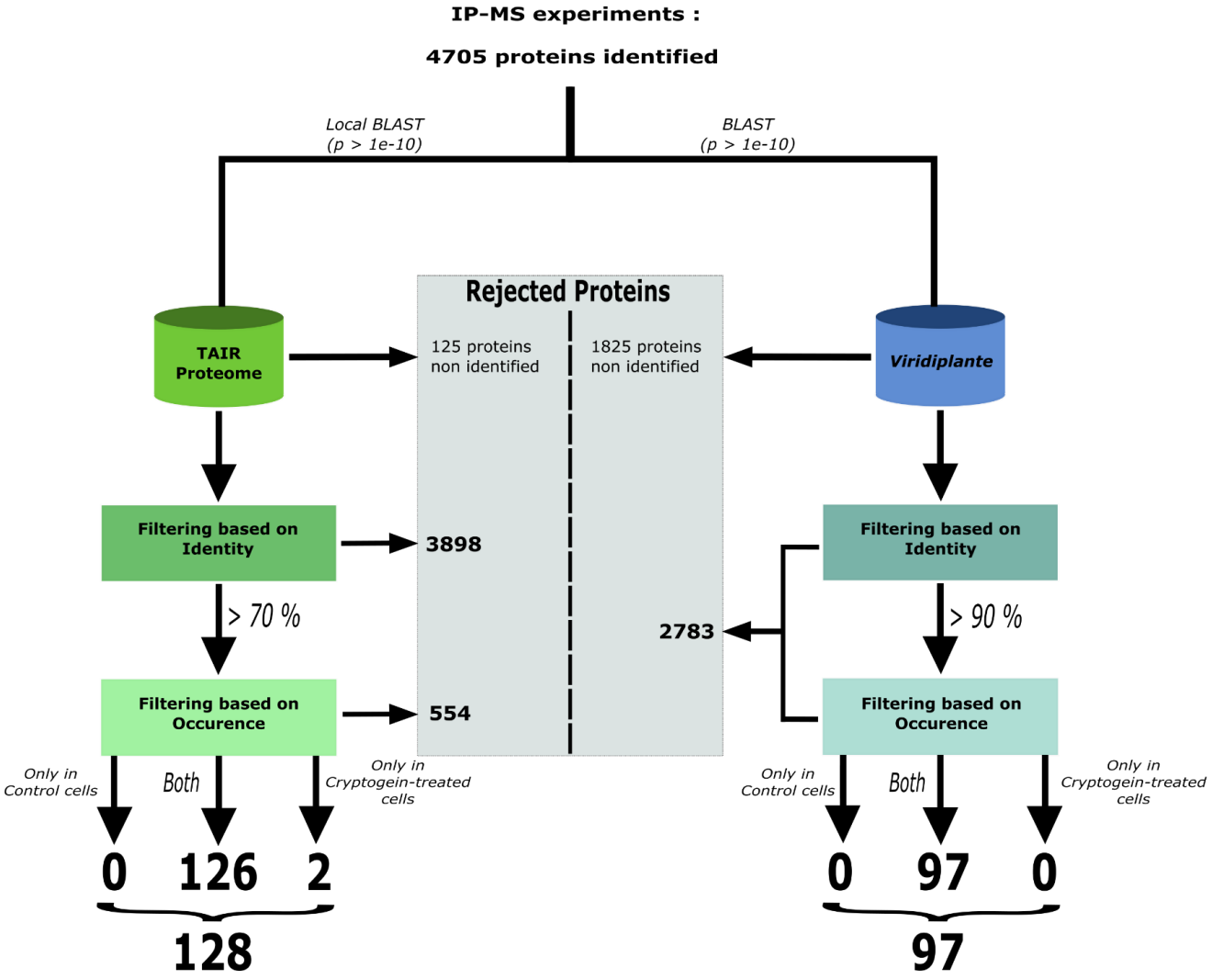
B) Simplified description of the different communities found into the plant CDC48 network. Each community is identified by a number, characterized by the number of nodes that compose the community and by a major node (i.e. the node with the highest degree).

C) Schematic focus on the cysteine methionine biosynthesis pathway (KEGG; ath00270) focusing on S-adenosyl L-methionine (red point). Protein partners of CDC48 are framed and the community in which the protein was found is specified. SAMS: S-adenosyl methionine synthase; MS1 : 5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase 1; SAHH1 : Adenosylhomocysteinase 1.

Figure S1 - Comparison of the protein classes belonging to the human, plant and yeast CDC48 interactomes. Pie charts represent the proportion of each protein class of the CDC48

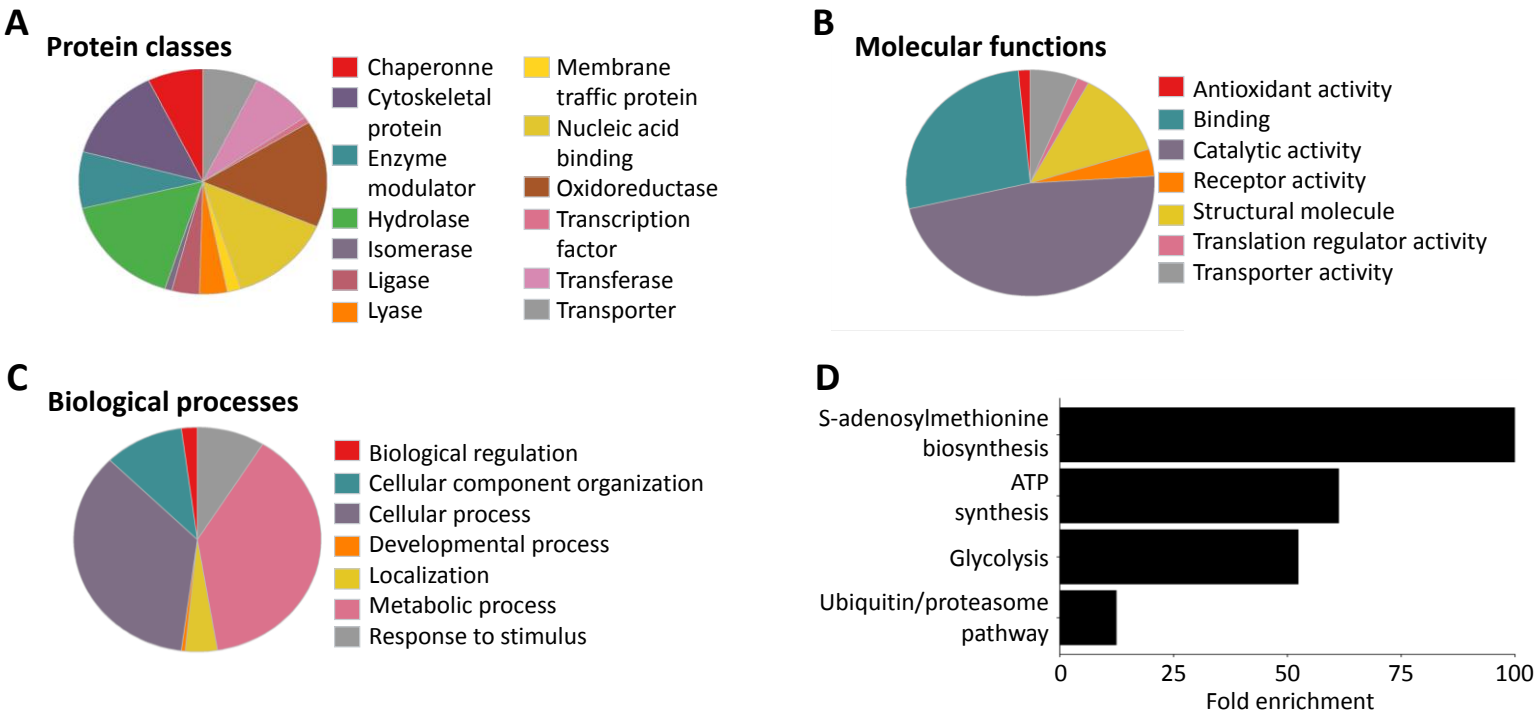
742 interactomes. For a given interactome, the number of classes is specified. The analysis was
743 performed thanks to the PANTHER database classification tool.
744
745 Figure S2 - Comparison of the molecular functions and biological processes of the human,
746 plant and yeast CDC48 partners.
747 A) Pie chart representing the molecular functions of CDC48 partners and their respective
748 proportion.
749 (B) Pie chart representing the biological processes in which the CDC48 partners are involved
750 and their respective proportion.
751 The analysis was performed thanks to the PANTHER database classification tool.
752
753

Figure 1



(Rosnoblet *et al.*, 2017)

Figure 2


A
Protein classes
Chaperonne
Cytoskeletal protein
Enzyme modulator
Hydrolase
Isomerase
Ligase
Lyase
Membrane traffic protein
Nucleic acid binding
Oxidoreductase
Transcription factor
Transferase
Transporter
B
Molecular functions
Antioxidant activity
Binding
Catalytic activity
Receptor activity
Structural molecule
Translation regulator activity
Transporter activity
C
Biological processes
Biological regulation
Cellular component organization
Cellular process
Developmental process
Localization
Metabolic process
Response to stimulus
D
S-adenosylmethionine biosynthesis
ATP synthesis
Glycolysis
Ubiquitin/proteasome pathway
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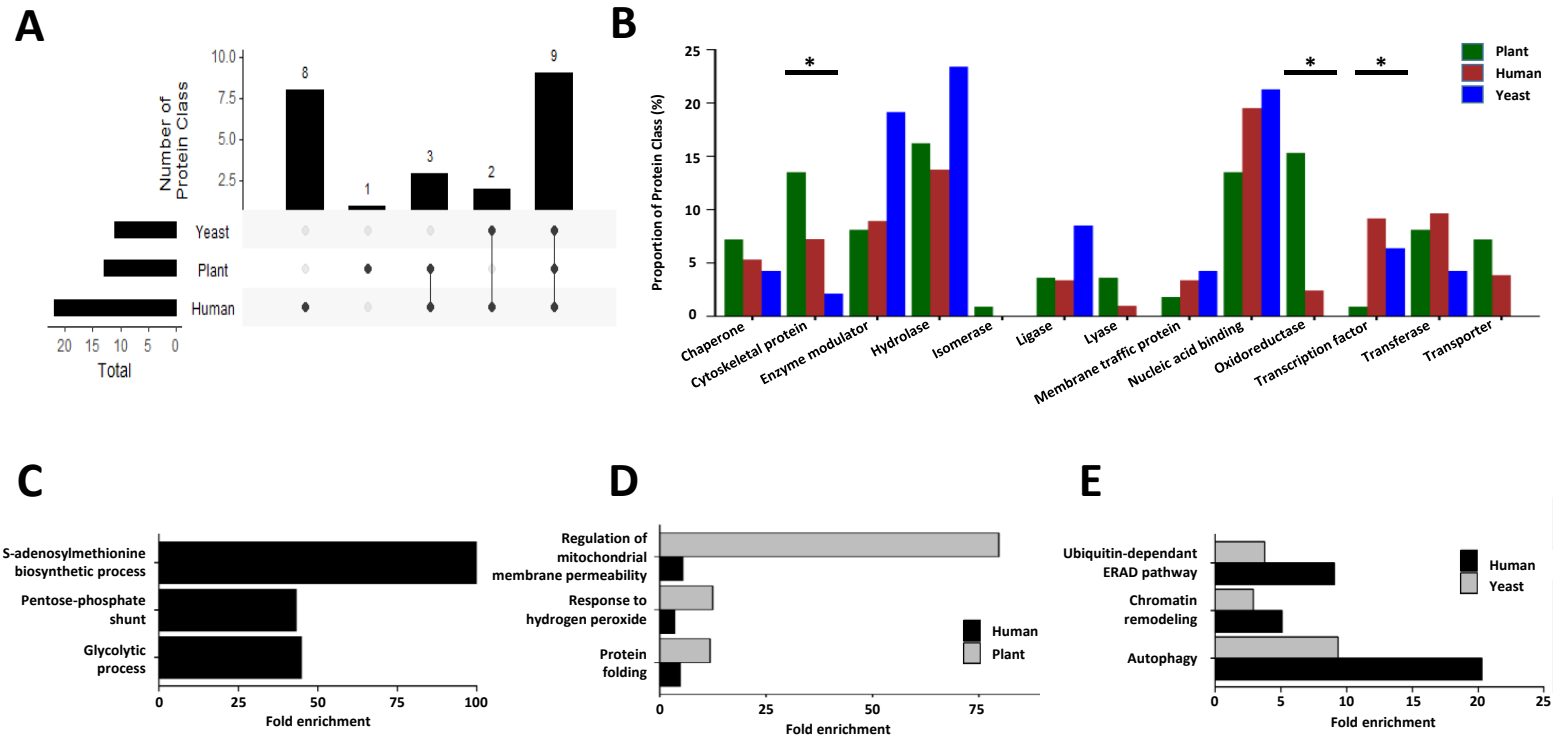
25
50
75
100
Fold enrichment

Figure 3


A
Number of Protein Class
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7.5
5.0
2.5
8
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3
2
9
Yeast
Plant
Human
20 15 10 5 0
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Proportion of Protein Class (%)
25
20
15
10
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*
*
Plant
Human
Yeast
Chaperone
Cytoskeletal protein
Enzyme modulator
Hydrolase
Isomerase
Ligase
Lyase
Membrane traffic protein
Nucleic acid binding
Oxidoreductase
Transcription factor
Transferase
Transporter
C
S-adenosylmethionine biosynthetic process
Pentose-phosphate shunt
Glycolytic process
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Fold enrichment
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Regulation of mitochondrial membrane permeability
Response to hydrogen peroxide
Protein folding
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Chromatin remodeling
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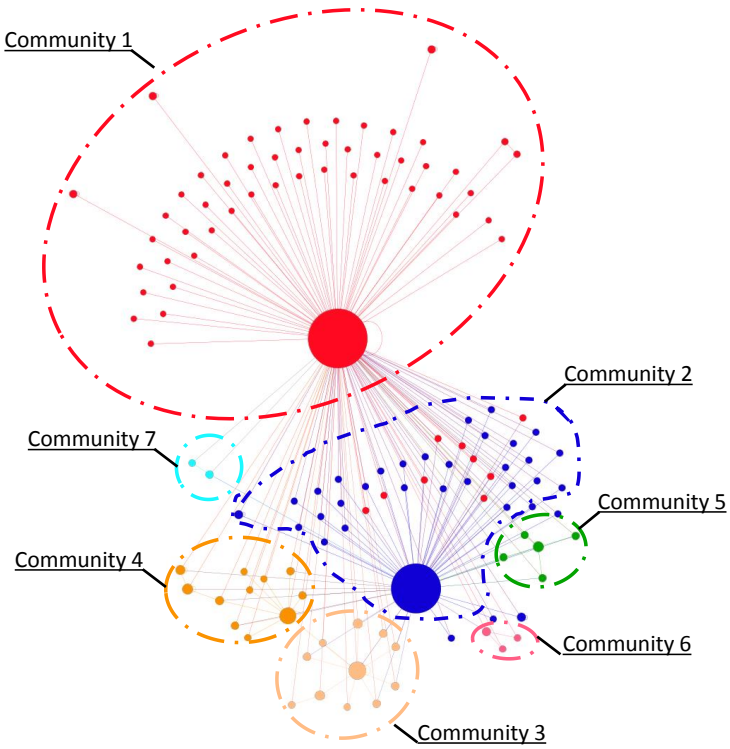
25
Fold enrichment

Figure 4

A



B

COMMUNITY	NUMBER OF NODES	MAJOR NODE
1	62	CDC48
2	35	UBQ3
3	11	GRF2
4	11	GRF8
5	5	RPN1A
6	3	CR88
7	2	IDH1

C

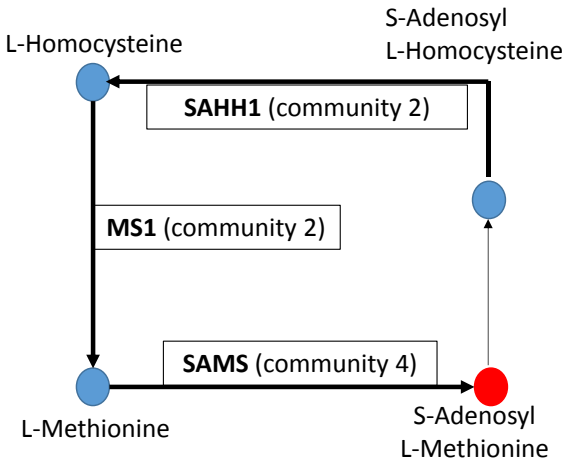


Tableau 1 List of CDC48 partners. Partners of CDC48 are separated according to functions. Stars (*) indicate proteins with orthologs among the Nicotiana partners. Crosses (ǂ) indicate partners that were only found during the cryptogein treatment.

TAIR ID	ENTRY NAME	Protein names
		CDC48
AT3g09840	CD48A_ARATH	Cell division control protein 48 homolog A *
		Proteasome related
AT1g20200	PSD3A_ARATH	26S proteasome non-ATPase regulatory subunit 3 A (RPN3-A) *
AT4g24820	PSMD6_ARATH	26S proteasome non-ATPase regulatory subunit 7 (RPN7)
AT2g20580	PSD2A_ARATH	26S proteasome non-ATPase regulatory subunit 1 A (RPN1-A)
AT5g58290	PRS6B_ARATH	26S proteasome regulatory subunit 3 (RPT3) *
AT1g45000	PS10B_ARATH	26S proteasome regulatory subunit 4 B (RPT4B) *
AT4g05050	UBQ11_ARATH	Polyubiquitin 11 *
AT5g03240	UBQ3_ARATH	Polyubiquitin 3 *
		14-3-3 proteins
AT1g78300	14332_ARATH	14-3-3-like protein GF14 omega *
AT2g42590	14339_ARATH	14-3-3-like protein GF14 mu *
AT5g65430	14338_ARATH	14-3-3-like protein GF14 kappa
		Tubulins and Actins
AT4g14960	TBA6_ARATH	Tubulin alpha-6 chain *
AT1g20010	TBB5_ARATH	Tubulin beta-5 chain *
AT1g50010	TBA2_ARATH	Tubulin alpha-2 chain
AT5g19780	TBA5_ARATH	Tubulin alpha-5 chain
AT5g62700	TBB3_ARATH	Tubulin beta-3 chain
AT5g12250	TBB6_ARATH	Tubulin beta-6 chain
AT2g29550	TBB7_ARATH	Tubulin beta-7 chain
AT5g23860	TBB8_ARATH	Tubulin beta-8 chain
AT3g12110	ACT11_ARATH	Actin-11 *
AT5g09810	ACT7_ARATH	Actin-7 *
AT3g53750	ACT3_ARATH	Actin-3
		ATP synthesis/Transport
ATCg00120	ATPA_ARATH	ATP synthase subunit alpha, chloroplastic (EC 3.6.3.14) *
AT5g08680	ATPBO_ARATH	ATP synthase subunit beta-3, mitochondrial (EC 3.6.3.14) *
AT2g07698	F4IMB5_ARATH	ATPase, F1 complex, alpha subunit protein *
ATMg01190	ATPAM_ARATH	ATP synthase subunit alpha, mitochondrial
ATCg00480	ATPB_ARATH	ATP synthase subunit beta, chloroplastic (EC 3.6.3.14)
AT2g33040	ATPG3_ARATH	ATP synthase subunit gamma, mitochondrial
AT5g13490	ADT2_ARATH	ADP,ATP carrier protein 2, mitochondrial
AT4g28390	ADT3_ARATH	ADP,ATP carrier protein 3, mitochondrial
		Biosynthesis of cellular compounds:
		Amino-acids

AT2g36880	METK3_ARATH	S-adenosylmethionine synthase 3 (EC 2.5.1.6) *
AT1g02500	METK1_ARATH	S-adenosylmethionine synthase 1 (EC 2.5.1.6)
AT4g01850	METK2_ARATH	S-adenosylmethionine synthase 2 (EC 2.5.1.6)
AT3g17390	METK4_ARATH	S-adenosylmethionine synthase 4 (EC 2.5.1.6)
AT5g17920	METE1_ARATH	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase 1 (EC 2.1.1.14) *
AT4g14880	CYSK1_ARATH	Cysteine synthase 1 (EC 2.5.1.47) *
AT4g13940	SAHH1_ARATH	Adenosylhomocysteinase 1 (EC 3.3.1.1) *
AT5g10240	ASNS3_ARATH	Glutamine-dependent asparagine synthetase 3 (EC 6.3.5.4) *
AT4g24830	ASSY_ARATH	Argininosuccinate synthase, chloroplastic (EC 6.3.4.5) *
AT2g19940	ARGC_ARATH	Probable N-acetyl-gamma-glutamyl-phosphate reductase, chloroplastic (EC 1.2.1.38) *
AT3g58610	ILV5_ARATH	Ketol-acid reductoisomerase, chloroplastic (EC 1.1.1.86)
AT4g13930	GLYC4_ARATH	Serine hydroxymethyltransferase 4 (EC 2.1.2.1)
		Proteins
AT5g60390	EF1A4_ARATH	Elongation factor 1-alpha 4 *
AT1g56070/AT1g56075	EF2_ARATH	Elongation factor 2 *
AT1g09640	EF1G1_ARATH	Probable elongation factor 1-gamma 1 *
AT3g13920	IF4A1_ARATH	Eukaryotic initiation factor 4A-1 (EC 3.6.4.13) *
AT4g02930	EFTM_ARATH	Elongation factor Tu, mitochondrial
AT4g26300	SYRM_ARATH	Arginine--tRNA ligase, chloroplastic/mitochondrial (EC 6.1.1.19) ǂ
AT1g29880	SYGM1_ARATH	Glycine--tRNA ligase, mitochondrial 1 (EC 6.1.1.14)
		Pyrimidines
AT3g54470	UMPS_ARATH	Uridine 5'-monophosphate synthase (EC 2.4.2.10) (EC 4.1.1.23) *
		Lipids
AT5g49460	ACLB2_ARATH	ATP-citrate synthase B-2 (EC 2.3.3.8)
AT5g35360	ACCC_ARATH	Biotin carboxylase, chloroplastic (EC 6.3.4.14)
		Chaperonne and related proteins
AT5g02500	MD37E_ARATH	Probable mediator of RNA polymerase II transcription subunit 37e *
AT5g42020	MD37F_ARATH	Mediator of RNA polymerase II transcription subunit 37f *
AT3g12580	MD37C_ARATH	Probable mediator of RNA polymerase II transcription subunit 37c
AT3g20050	TCPA_ARATH	T-complex protein 1 subunit alpha
AT3g11830	TCPH_ARATH	T-complex protein 1 subunit eta
AT3g03960	TCPQ_ARATH	T-complex protein 1 subunit theta
AT5g56000	HS904_ARATH	Heat shock protein 90-4 *
AT5g56030	HS902_ARATH	Heat shock protein 90-2
AT2g04030	HS905_ARATH	Heat shock protein 90-5, chloroplastic
AT4g24190	ENPL_ARATH	Endoplasmin homolog
AT5g09590	HSP7J_ARATH	Heat shock 70 kDa protein 10, mitochondrial
AT1g79930	HSP7O_ARATH	Heat shock 70 kDa protein 14
AT1g79920	HSP7P_ARATH	Heat shock 70 kDa protein 15
AT1g16030	HSP7E_ARATH	Heat shock 70 kDa protein 5
AT5g49910	HSP7G_ARATH	Heat shock 70 kDa protein 7, chloroplastic
AT3g23990	CH60A_ARATH	Chaperonin 60, mitochondria

AT3g13470	CPNB2_ARATH	Chaperonin 60 subunit beta 2, chloroplastic
		Enzymes, espacially glycolysis related
AT5g41670	6PGD2_ARATH	6-phosphogluconate dehydrogenase, decarboxylating 2, chloroplastic (EC 1.1.1.44)
AT3g02360	6GPD3_ARATH	6-phosphogluconate dehydrogenase, decarboxylating 3 (EC 1.1.1.44)
AT2g05710	ACO3M_ARATH	Aconitate hydratase 3, mitochondrial (EC 4.2.1.3)
AT5g43940	ADHX_ARATH	Alcohol dehydrogenase class-3 (EC 1.1.1.1)
AT1g77120	ADH1_ARATH	Alcohol dehydrogenase class-P (EC 1.1.1.1) *
AT3g48000	AL2B4_ARATH	Aldehyde dehydrogenase family 2 member B4, mitochondrial (EC 1.2.1.3) *
AT1g23800	AL2B7_ARATH	Aldehyde dehydrogenase family 2 member B7, mitochondrial (EC 1.2.1.3)
AT4g26270	PFKA3_ARATH	ATP-dependent 6-phosphofructokinase 3 (EC 2.7.1.11)
AT2g36530	ENO2_ARATH	Bifunctional enolase 2/transcriptional activator (EC 4.2.1.11) *
AT1g65930	ICDHC_ARATH	Cytosolic isocitrate dehydrogenase (EC 1.1.1.42) *
AT1g74030	ENO1_ARATH	Enolase 1, chloroplastic (EC 4.2.1.11)
AT4g38970	ALFP2_ARATH	Fructose-bisphosphate aldolase 2, chloroplastic (EC 4.1.2.13) *
AT2g21330	ALFP1_ARATH	Fructose-bisphosphate aldolase 1, chloroplastic (EC 4.1.2.13)
AT2g01140	ALFP3_ARATH	Fructose-bisphosphate aldolase 3, chloroplastic (EC 4.1.2.13)
AT2g36460	ALFC6_ARATH	Fructose-bisphosphate aldolase 6, cytosolic (EC 4.1.2.13)
AT1g74470	CHLP_ARATH	Geranylgeranyl diphosphate reductase, chloroplastic (EC 1.3.1.83) *
AT5g40760	G6PD6_ARATH	Glucose-6-phosphate 1-dehydrogenase, isoform 2, cytoplasmic (EC 1.1.1.49) *
AT1g12900	G3PA2_ARATH	Glyceraldehyde-3-phosphate dehydrogenase GAPA2, chloroplastic (EC 1.2.1.13) *
AT3g04120	G3PC1_ARATH	Glyceraldehyde-3-phosphate dehydrogenase GAPC1, cytosolic (EC 1.2.1.12) *
AT1g13440	G3PC2_ARATH	Glyceraldehyde-3-phosphate dehydrogenase GAPC2, cytosolic (EC 1.2.1.12) *
AT1g16300	G3PP2_ARATH	Glyceraldehyde-3-phosphate dehydrogenase GAPCP2, chloroplastic (EC 1.2.1.12)
AT4g35260	IDH1_ARATH	Isocitrate dehydrogenase [NAD] regulatory subunit 1, mitochondrial (EC 1.1.1.41) *
AT5g14590	ICDHP_ARATH	Isocitrate dehydrogenase [NADP], chloroplastic/mitochondrial (EC 1.1.1.42) *
AT5g03290	IDH5_ARATH	Isocitrate dehydrogenase [NAD] catalytic subunit 5, mitochondrial (EC 1.1.1.41)
AT1g04410	MDHC1_ARATH	Malate dehydrogenase 1, cytoplasmic (EC 1.1.1.37) *
AT3g59970	MTHR1_ARATH	Methylenetetrahydrofolate reductase 1 (EC 1.5.1.20)
AT2g44160	MTHR2_ARATH	Methylenetetrahydrofolate reductase 2 (EC 1.5.1.20) *
AT5g25880	MAOP3_ARATH	NADP-dependent malic enzyme 3 (EC 1.1.1.40)
AT1g56190	PGKH2_ARATH	Phosphoglycerate kinase 2, chloroplastic (EC 2.7.2.3) *
AT1g79550	PGKY3_ARATH	Phosphoglycerate kinase 3, cytosolic (EC 2.7.2.3) *
AT1g70730	PGMC2_ARATH	Probable phosphoglucomutase, cytoplasmic 2 (EC 5.4.2.2) *
AT1g76550	PFPA2_ARATH	Pyrophosphate--fructose 6-phosphate 1-phosphotransferase subunit alpha 2
AT3g52990	Q94KE3_ARATH	Pyruvate kinase (EC 2.7.1.40) *
ATCg00490	RBL_ARATH	Ribulose bisphosphate carboxylase large chain (EC 4.1.1.39) *
AT5g15490	UGDH3_ARATH	UDP-glucose 6-dehydrogenase 3 (EC 1.1.1.22) *
AT3g29360	UGDH2_ARATH	UDP-glucose 6-dehydrogenase 2 (EC 1.1.1.22)
		ROS processing system
AT1g07890	APX1_ARATH	L-ascorbate peroxidase 1, cytosolic (EC 1.11.1.11) *
AT4g35090	CATA2_ARATH	Catalase-2 (EC 1.11.1.6) *
		RNA processing
AT2g27040	AGO4_ARATH	Protein argonaute 4 *

AT5g65260	PABN2_ARATH	Polyadenylate-binding protein 2 *
AT4g39260	RBG8_ARATH	Glycine-rich RNA-binding protein 8 *
AT3g51800	EBP1_ARATH	ERBB-3 BINDING PROTEIN 1‡
		Trafficking
AT3g11130	CLAH1_ARATH	Clathrin heavy chain 1 *
AT1g52360	COB22_ARATH	Coatomer subunit beta'-2
		Transport
AT4g17170	RAB1C_ARATH	Ras-related protein RABB1c *
AT3g12390	NACA1_ARATH	Nascent polypeptide-associated complex subunit alpha-like protein 1 *
AT1g78900	VATA_ARATH	V-type proton ATPase catalytic subunit A (EC 3.6.3.14)
AT5g14040	MPCP3_ARATH	Mitochondrial phosphate carrier protein 3, mitochondrial
AT2g31660	SAD2_ARATH	Importin beta-like
		Other
AT5g15650	RGP2_ARATH	UDP-arabinopyranose mutase 2 (EC 5.4.99.30)
AT1g78570	RHM1_ARATH	Trifunctional protein (EC 4.2.1.76) (EC 1.1.1.-) (EC 5.1.3.-)
AT5g03300	ADK2_ARATH	Adenosine kinase 2 (EC 2.7.1.20) *
AT5g01410	PDX13_ARATH	Pyridoxal 5'-phosphate synthase subunit PDX1.3 (EC 4.3.3.6) *
AT1g04850	Q9MAT3_ARATH	F13M7.16 protein (Ubiquitin-associated (UBA)/TS-N domain-containing protein)
AT2g39730	RCA_ARATH	Ribulose bisphosphate carboxylase/oxygenase activase, chloroplastic *
AT1g65260	VIPP1_ARATH	Membrane-associated protein VIPP1, chloroplastic
AT3g43190	SUS4_ARATH	Sucrose synthase 4 (EC 2.4.1.13) *
AT3g03250	UGPA2_ARATH	UTP--glucose-1-phosphate uridylyltransferase 2 (EC 2.7.7.9) *
AT5g47720	THIC2_ARATH	Probable acetyl-CoA acetyltransferase, cytosolic 2 (EC 2.3.1.9) *