

Research

Evaluation and classification of RING-finger domains encoded by the *Arabidopsis* genome

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Abstract

Background: In computational analysis, the RING-finger domain is one of the most frequently detected domains in the *Arabidopsis* proteome. In fact, it is more abundant in *Arabidopsis* than in other eukaryotic genomes. However, computational analysis might classify ambiguous domains of the closely related PHD and LIM motifs as RING domains by mistake. Thus, we set out to define an ordered set of *Arabidopsis* RING domains by evaluating predicted domains on the basis of recent structural data.

Results: Inspection of the proteome with a current InterPro release predicts 446 RING domains. We evaluated each detected domain and as a result eliminated 59 false positives. The remaining 387 domains were grouped by cluster analysis and according to their metal-ligand arrangement. We further defined novel patterns for additional computational analyses of the proteome. They were based on recent structural data that enable discrimination between the related RING, PHD and LIM domains. These patterns allow us to predict with different degrees of certainty whether a particular domain is indeed likely to form a RING finger.

Conclusions: In summary, 387 domains have a significant potential to form a RING-type cross-brace structure. Many of these RING domains overlap with predicted PHD domains; however, the RING domain signature mostly prevails. Thus, the abundance of PHD domains in *Arabidopsis* has been significantly overestimated. Cluster analysis of the RING domains defines groups of proteins, which frequently show significant similarity outside the RING domain. These groups document a common evolutionary origin of their members and potentially represent genes of overlapping functionality.

Background

The recent completion of the *Arabidopsis thaliana* genome sequence and its accessibility in annotated form [1] marks an essential breakthrough for basic and applied plant science. Extensive bioinformatics analysis, using both extrinsic and intrinsic data, initially detected 25,498 genes within the *Arabidopsis* genome. Around 69% of the corresponding proteins could be classified according to their sequence similarity to

proteins of known function in plants and other organisms. Approximately 51% of the genes contain a functional domain detectable by InterPro. InterPro has proven to be especially powerful for functional domain detection [1-3].

One of the most abundant domains detected in the *Arabidopsis* proteome is the RING-finger domain, which was found 365 times in the initial characterization of the

genome [1]. In fact, 1.42% of *Arabidopsis* proteins contain a RING-domain signature. Thus, it is overrepresented in *Arabidopsis* as compared to other complete eukaryotic genomes (*Drosophila melanogaster*, *Caenorhabditis elegans* and *Saccharomyces cerevisiae*), which contain 0.7-0.75% of RING-domain proteins. To date, the significance of this observation remains unclear.

The RING domain was originally named after the acronym for the first protein it was found in, encoded by the *Really Interesting New Gene* [4,5]. The motif is related to the zinc-finger domain; however, zinc fingers consist of two pairs of zinc ligands coordinately binding one zinc ion, whereas RING fingers consist of four pairs of ligands binding two

ions. Two other motifs that consist of four metal-ligand pairs binding two zinc ions are related to the RING finger - the PHD (plant homeodomain) [6] and LIM (Lin11/Isl-1/Mec-3) [7] domains (Figure 1).

The RING-domain structure has been resolved at atomic resolution for three proteins - promyelotic leukemia protein (PML), equine herpes virus protein (IEEVH) and the human recombination protein RAG1 [8-10]. These studies revealed that the RING domain forms a distinct so-called cross-brace structure, in which metal-ligand pairs 1 and 3 coordinate to bind one zinc ion, and pairs 2 and 4 bind the second one (Figure 1a). Also, the structures of the LIM-domain proteins CRP1, CRIP and CRP2 [11-13] have been resolved and indicate that LIM domains

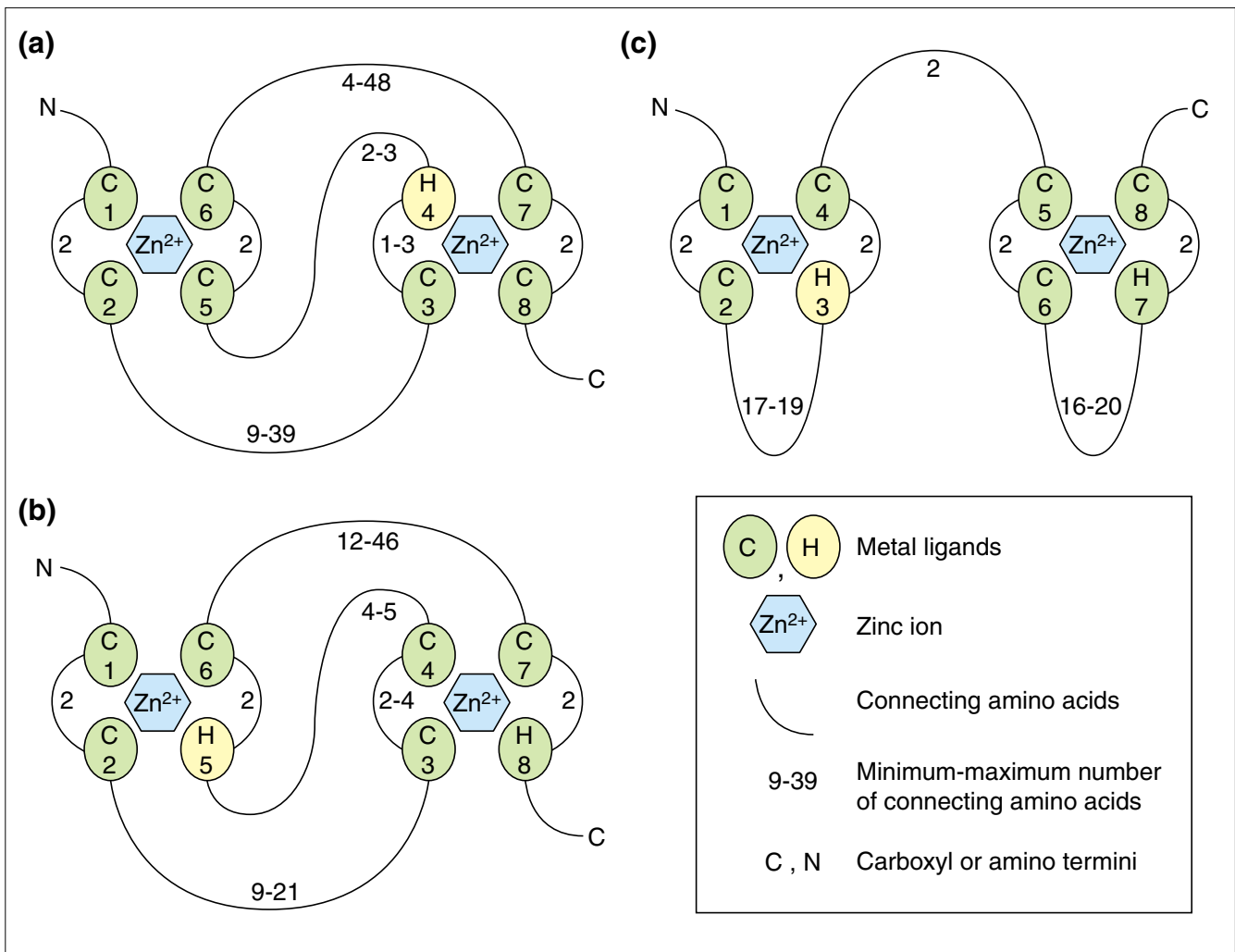


Figure 1
 Schematic presentation of the structure of prototypical RING, PHD and LIM domains. The metal-ligand residues, either cysteine (C) or histidine (H), are shown as numbered spheres. Two pairs of metal ligands coordinate one zinc ion (hexagon). The numbers next to the loops connecting the metal-ligand residues indicate the minimum and maximum number of loop residues. **(a)** The structure of a RING domain (RING-HC type). The metal-ligand pairs 1 and 3 coordinate one zinc ion, while pairs 2 and 4 coordinate the second one in a so-called cross-brace arrangement. **(b)** The structure of a PHD domain reveals a cross-brace arrangement similar to the RING domain. **(c)** The LIM domain structure is distinct in its consecutive zinc ligation scheme: the first zinc ion is coordinated by the metal-ligand pairs 1 and 2, while the second ion is coordinated by pairs 3 and 4.

behave like a double zinc finger, coordinating two zinc ions with the two consecutive pairs of ligands (Figure 1c). Finally, the recent solution of the structure of the PHD domain of KAP-1 [14] demonstrates that the zinc-ligation scheme of the PHD domain is similar to the RING domain; that is, it folds into a cross-brace structure (Figure 1b).

In functional terms, the RING domain can basically be considered a protein-interaction domain, and RING-finger proteins have been implicated in a range of diverse biological processes and biochemical activities, from transcriptional and translational regulation to targeted proteolysis [15-17]. For several RING proteins a biochemical ubiquitin ligase activity has been observed [18,19]. Thus it has been suggested that the abundance of RING proteins in *Arabidopsis* might reflect a bias towards target-specific proteolysis as a means of controlling gene activity. However, in most cases a RING domain by itself is not sufficient for ubiquitin ligase activity and the additional structural features required are not known. Thus, to date it remains unclear how many of the RING proteins encoded in the *Arabidopsis* genome could indeed be involved in protein degradation.

In this in-depth study, we evaluate and classify RING domain proteins of *Arabidopsis* by computational analyses as well as manual curation. We present a set of *Arabidopsis* RING domains, which we classify into related clusters and sort according to their potential to form a RING-type cross-brace structure on the basis of recent results from structural analyses.

Results and discussion

InterPro analysis of the *Arabidopsis* proteome for RING-domain proteins

In an initial characterization of the *Arabidopsis* proteome [1] by InterPro analysis (release 1.0) [2] a total of 365 RING domains were detected. We searched the proteome for RING domains again, using an updated version of InterPro (release 3.1). In total, our analysis retrieved 446 domains. An overall percentage of 10-15% of erroneously assigned exons has been estimated for the proteome. However, exons that contain functional domains, and which are therefore detectable and adjustable via similarity-based methods, are expected to have significantly fewer wrong assignments. We therefore have confidence in the detected number of RING-finger-containing proteins, although a deviation in the lower single-digit percentage range cannot be excluded.

We evaluated the first-pass computational analysis and manually annotated all detected domains according to a set of criteria that would qualify them as RING domains. All our results are organized on two websites that cross-reference each other [20,21]. Here we present an overview and a summary of the results; corresponding details and supplementary materials can be found on the websites.

Criteria for RING domains

Several of the detected domains did not represent a full-length RING domain. We inspected them in detail and attempted to complete them using the adjacent sequences in the respective proteins. This proved successful in a number of cases; in several others, however, it was not possible. We also noticed that in some domains the conserved spacing of the metal ligands was lacking, indicative of probable false-positive detection. In fact, in addition to programs relying on defined strings, modules not based on defined patterns (such as PfamHMM, which is based on hidden Markov models) are part of InterPro. As a result, domains not containing the defined patterns might have been detected by InterPro as RING domains. Thus, we inspected each of the 446 initially detected RING domains in detail, in order to eliminate false positives from the analysis set.

To verify the RING domains, we defined a set of criteria based on well characterized examples of RING-domain proteins. First, we required domains to contain at least seven of the full complement of eight metal ligands. Second, the metal-ligand residues had to correspond to the RING pattern, either to the prototypical RING pattern (metal ligand 4 is histidine, all others are cysteine, 'RING-HC', see below) or the frequent RING-H2 pattern (metal ligands 4 and 5 histidine, all others cysteine). Third, the spacing of the core residues (metal ligands 3 to 6) had to be conserved. These criteria leave room for subgroups of RING-finger structures in which the spacing between positions 7 and 8 is different from the generally conserved two residues [22], and for cases in which a metal ligand is missing, mostly at the seventh or eighth position. Finally, we also allowed additional deviations from the canonic criteria, which ensure that known variant-type RING domains [23] are included in our set. These modifications are metal ligand substitutions that are observed in a few well characterized RING domains, such as those in MDM2, mouse c-Cbl, Rbx1 and CART1. They include threonine for cysteine substitutions at metal ligand positions 1 or 3 and aspartate for cysteine substitution at metal ligand 8.

We did not allow for similar substitutions at other positions, as the ligands might not be interchangeable in every position [14]. However, we carried out additional computational analyses with more relaxed patterns. For instance, if metal ligand positions 1, 3, 5 and 8 are simultaneously allowed to be encoded by either cysteine, histidine, threonine or aspartate (see above), a total of 81 additional domains in 79 proteins are found. Although these domains are not detectable by InterPro analysis, they might have the potential to encode novel RING-domain variants. However, several LIM domains are included in this set. Moreover, the corresponding RING arrangement was unclear in most other cases and might represent distantly related motifs, such as the U-box [24]. Thus we did not include these additional domains in our proper set.

Other domains in RING proteins

A complete InterPro analysis on the protein set retrieved with the RING motif revealed other domains present in these proteins. The RING domain is closely related to the LIM and PHD domains, and 141 of the RING-domain proteins are also reported to carry a PHD domain. We inspected these predicted PHD domains and the vast majority of the respective metal ligands overlap with the detected RING domains. However, in most cases the RING-domain signature prevails, as some highly conserved residues characteristic of PHD domains are mostly missing. In fact, only in three cases did we clearly favor a PHD-domain architecture, and these domains were eliminated from our set. This means that the *Arabidopsis* proteome possibly contains 138 fewer PHD domains than detected. Thus, because of the overlap in the RING- and PHD-domain signatures, the frequency of the PHD domain has initially been significantly overestimated.

After the PHD domain, bipartite nuclear-localization signals were the second most frequently detected domain (63 times) in our RING-domain protein set. Most other domains are much less frequent however (< 10 times), and some combinations are obviously absent. For instance, the RING/B-box/coiled-coil protein family found in several eukaryotes seems to be absent from *Arabidopsis*.

Elimination of false positives

On the basis of our criteria above, the metal-ligand arrangement of every one of the initial 446 domains was re-inspected. We noted that some proteins with a generally high content of cysteine and histidine residues represent false positives. Indeed, that is the case for a group of five cellulose synthases, which contain several zinc-finger domains. We eliminated these from our set.

We also eliminated 41 additional domains in which at least two metal ligands or possible substituting residues are missing, and 10 domains in which the spacing of the core residues (metal ligands 3 to 6) did not satisfy the criteria. Thus, in total, 59 probable false-positive domains have been eliminated from our initial set (Table 1).

Classification of RING domains

We classified the remaining set of 387 domains according to metal-ligand arrangement. The originally described RING domains were characterized by a histidine at metal-ligand position 4. We have termed the domains in our set with a corresponding arrangement RING-HC domains. We found 118 domains of this type in 111 different proteins. However, the cysteine usually present at metal-ligand position 5 is frequently substituted by a histidine as well, and we identify these domains as RING-H2 domains. Of this type of domain, 215 were found in 214 proteins. The remaining 54 domains, in which not all of the metal ligands were either cysteine or histidine, but where one metal ligand is missing or substituted according to our criteria described above, were classified as RING-variant domains [23].

Derivation of additional patterns for computational analysis

An inherent problem with the computational detection of RING-finger domains is their relatedness to the PHD domain (see above). This ambiguity seems to be due to a lack of structural determinants that separate a given domain in one group or the other. Recently, the first solution structure for a PHD domain has been obtained [14]. Its comparison with related structures revealed some key features that separate LIM, PHD and RING domains.

The LIM domain is clearly set apart from the two others, with a more conserved spacing and conserved hydrophobic residues not found in RING or PHD domains. Among the conserved hydrophobic residues, one is located in front of metal ligand 3 and one after metal ligand 4, and these features result in a zinc ligation by consecutive metal ligand pairs. By contrast, hydrophobic residues in front of metal ligand 5 and after metal ligand 6 seem to result in a cross-brace arrangement, which is observed in RING and PHD domains. Despite this commonality, two features separate RING and PHD domains. First, the loop between metal ligands 4 and 5 can be up to five residues in PHD domains, rather than only up to three in RING domains. Second, in PHD domains the residue two positions in front of metal ligand 7 is an aromatic residue, which alters the hydrophobic core of the domain and thus its structural characteristics.

On the basis of the above data, we defined four patterns that specify a RING domain with increasing stringency (Table 2). In our first pattern (Stringent 1) we required that all metal ligands are present, according to our criteria outlined above, and that the position two residues in front of metal ligand 7 is not an aromatic amino acid. These criteria were satisfied by 324 domains, whereas it was not the case for 63 domains. Failure to match the criteria is mainly the result of a missing or substituted metal ligand; that is, these domains are classified as RING variants. However, among those 63 domains, 13 carry an aromatic residue two positions in front of metal ligand 7 and we thus consider them unlikely to form a RING domain. Rather, they are structurally more similar to PHD domains. Therefore, of the 141 PHD domains predicted in our initial RING-domain set (see above) as few as 16 might indeed represent a PHD structure.

In our second pattern (Stringent 2) we added another criterion; we required the positions in front of metal ligand 5 and after metal ligand 6 to be hydrophobic or serine or threonine, which have been observed in some well characterized RING proteins [14]. Hydrophobic residues at these positions are critical for cross-brace structure formation. Thirty more domains failed this test. However, the remaining 294 domains can be considered fairly certain to form a RING structure.

Next (Stringent 3), we abolished the acceptance of threonine or serine after metal ligand 6, as these residues are

Table 1

Proteins with false-positive detected RING domains that were eliminated from the dataset

Protein ID	Reason	Remarks
At1g02860	Wrong ligand spacing	Spacer between metal ligands 2 and 3 too long
At1g05890	Wrong ligand spacing	Protein contains one RING-variant domain
At1g09060	Incomplete	
At1g10170	PHD domain	
At1g55530	Incomplete	Protein contains one RING-H2 domain
At1g60360	Incomplete	Protein contains one RING-H2 domain
At1g62310	Incomplete	
At1g74870	Incomplete	
At2g28530	Incomplete	
At2g31770	Wrong ligand spacing	Either wrong spacing or incomplete
		Protein contains one RING-variant domain
At2g31780	Wrong ligand spacing	Either wrong spacing or incomplete
		Protein contains one RING-variant domain
At2g39720	Incomplete	Protein contains one RING-H2 domain
At2g42170	Wrong ligand spacing	Pseudogene
At2g44330	Incomplete	Two false-positive motifs
		Protein contains one RING-H2 domain
At3g05870	Incomplete	Protein contains one RING-H2 domain
At3g06330	Wrong ligand spacing	
At3g07610	Incomplete	
At3g08020	PHD domain	
At3g18290	Incomplete	Protein contains one RING-H2 domain
At3g19910	Incomplete	
At3g19950	Incomplete	Protein contains one RING-H2 domain
At3g23060	Incomplete	Two false-positive motifs
At3g42830	Incomplete	Rbx1-like
		Protein contains one RING-variant domain
At3g45470	Wrong ligand spacing	Protein contains one RING-variant domain
At3g45540	Incomplete	Protein contains one RING-variant domain
At3g45630	Incomplete	
At3g48070	Wrong ligand spacing	
At3g52100	PHD domain	
At3g60080	Incomplete	Protein contains one RING-H2 domain
At3g62970	Incomplete	Protein contains one RING-H2 domain
At4g00070	Incomplete	
At4g01020	Incomplete	Protein contains one RING-HC and One RING-variant domain
At4g10940	Incomplete	
At4g12150	Incomplete	Protein contains one RING-variant domain, Which is part of a RING-H2 cluster
At4g26400	Incomplete	Protein contains one RING-H2 domain
At4g37880	Incomplete	
At4g39350	Wrong ligand spacing	Cellulose synthase
At5g05170	Wrong ligand spacing	Cellulose synthase
At5g09870	Wrong ligand spacing	Cellulose synthase
At5g10370	Incomplete	Protein contains one RING variant domain
At5g15790	Incomplete	Protein contains one RING-H2 domain
At5g17420	Wrong ligand spacing	Cellulose synthase
At5g23110	Incomplete	Protein contains one RING-HC domain
At5g25560	Incomplete	Protein contains one RING-H2 domain
At5g28340	Incomplete	

Table 1 (continued)

Protein ID	Reason	Remarks
At5g38070	Wrong ligand spacing	
At5g45290	Incomplete	
At5g47430	Incomplete	
At5g52140	Incomplete	
At5g52150	Incomplete	
At5g56340	Incomplete	Protein contains one RING-H2 domain
At5g58410	Wrong ligand spacing	
At5g62910	Wrong ligand spacing	
At5g63740	Incomplete	
At5g63750	Incomplete	<i>Drosophila</i> Ariadne-like
		Protein contains one RING-variant domain
At5g63970	Incomplete	
At5g64740	Wrong ligand spacing	Cellulose synthase

To eliminate false-positive RING domains detected through computational analysis, the complete dataset was curated and false positives were eliminated. The first column shows proteins in which false-positive RING domains were detected. The principal reason for their elimination from the dataset is given in the second column. 'Incomplete', at least two metal ligands are missing; 'wrong ligand spacing', the spacing between the prospective metal ligands is not conserved; 'PHD domain', the domain has a PHD rather than a RING structure. See text for additional criteria for RING domains. Alternative gene names and other remarks are given in the third column. For instance, many of the proteins contain an independent, verified RING domain and are thus also listed in Table 3.

rarely found in this position in well characterized RING proteins. Ten more domains did not comply with this requirement.

Finally, in our most stringent pattern (Stringent 4) we not only excluded aromatic residues from position 2 in front of metal ligand 7, but also other hydrophobic residues. An additional 55 domains from our set did not match this criterion. However, the 229 domains fulfilling these criteria can be considered to form a RING structure with near certainty.

Clustering of related RING domains

We sought to define groups of related RING-domain proteins beyond their classification by metal-ligand arrangement. However, it turned out that, at least in part, RING-domain proteins strongly deviate from each other outside the conserved domain. Thus it is not feasible to relate them using conventional phylogenetic methods. To circumvent this problem, the isolated RING-finger domains were used instead for further analysis. However, bootstrap values were again too low to reliably relate RING domains using phylogenetic methods. Therefore, a single-linkage-clustering method was applied to obtain clusters of related RING domains. We sorted our set on the basis of similarity restricted to the RING domains and excluding non-conserved amino- and carboxy-terminal parts of the respective

Table 2**Conventional and novel motif signatures used to identify RING domains in this study**

InterPro, RING-HC	C3HC4 long: C-X₂-C-X_{9,39}-C-X_{1,3}-H-X_{2,3}-C-X₂-C-X_{4,48}-C-X₂-C and short: C-X-H-X-[LIVMFY]-C-X₂-C-[LIVMYA]
InterPro, RING-H2	C3H2C3 long: C-X₂-C-X_{9,39}-C-X_{1,3}-H-X_{2,3}-H-X₂-C-X_{4,48}-C-X₂-C and short: C-X-H-X-[LIVMFY]-H-X₂-C-[LIVMYA]
Stringent 1	[CT]-X₂-C-X_{9,39}-[CT]-X_{1,3}-H-X_{2,3}-[CH]-X₂-C-X_{2,46}-[KRCHDESTQNGPLVIMA]-X-C-X₂-[CD]
Stringent 2	[CT]-X₂-C-X_{9,39}-[CT]-X_{1,3}-H-X_{1,2}-[WFYLVIMAST]-[CH]-X₂-C-[WFYLVIMAST]-X_{1,45}-[KRCHDESTQNGPLVIMA]-X-C-X₂-[CD]
Stringent 3	[CT]-X₂-C-X_{9,39}-[CT]-X_{1,3}-H-X_{1,2}-[WFYLVIMAST]-[CH]-X₂-C-[WFYLVIMA]-X_{1,45}-[KRCHDESTQNGPLVIMA]-X-C-X₂-[CD]
Stringent 4	[CT]-X₂-C-X_{9,39}-[CT]-X_{1,3}-H-X_{1,2}-[WFYLVIMAST]-[CH]-X₂-C-[WFYLVIMA]-X_{1,45}-[KRCHDESTQNGP]-X-C-X₂-[CD]

Domain signatures used to identify RING domains in the *Arabidopsis* proteome. The signatures used by the InterPro package as well as the novel patterns defined by us (Stringent 1-Stringent 4) on the basis of recent structural results are given. Metal ligands are in bold. For details see text.

proteins. A BLAST analysis [25] using a cut-off value of 10^{-15} was chosen to define meaningful similarities (Table 3). This analysis resulted in the definition of 54 clusters of RING domains (that is, two or more similar domains), in which 295 domains are grouped. Notably, with only one exception (cluster 2.8), all the RING-domain clusters only contain members from the same respective class of metal-ligand arrangement; that is, prototypical RING-HC domains are only found in clusters with other RING-HC domains. This finding confirms both the significance of our clustering and of the group definition described above. Of the 54 clusters, 28 consist of a pair of domains, whereas clusters with multiple domains contain up to 75 different domains. However, with the exception of the large clusters 2.1 (75 domains) and 2.2 (26 domains) found in the prototypical RING-H2 class, most clusters contain fewer than ten domains.

Redundancy of clustered proteins

To investigate overall protein similarities and reveal potential functional redundancy between proteins of a given cluster, we decided to produce alignments of the full-length proteins by ClustalW analysis [26]. Links to these alignments are provided with each cluster on our web page. Overall similarities vary between clusters. For instance, the 75 domains of cluster 2.1 contain several members of a RING-H2 family that has been described [27,28]. The proteins in this cluster are generally short, with only very little additional sequence outside the RING domain, and are highly similar to each other. Thus, any functional overlaps could already be contained in the RING domain itself. Other clusters, for example 2.6 and 2.8, consist of genes that are derived from tandem duplication.

In numerous other cases, however, the full-length alignments frequently reveal additional similarities outside the RING domain. For instance, cluster 1.1 includes the RMA1 protein, which has been shown to be a membrane-bound ubiquitin ligase [29]. The proteins in this cluster show some sequence similarity besides the RING domain and share additional features, such as a transmembrane anchor. Thus, it seems likely that these other proteins might also be

membrane-bound ubiquitin ligases. Another interesting group is cluster 2.2, which comprises 26 domains. The corresponding proteins also contain significant similarity in a stretch amino-terminal to the RING domain. Moreover, subgroups of proteins within the cluster even display additional similarity in the more distal amino-terminal regions. Two of the proteins in this cluster, AIP2 and CIP8, have been described [30,31]. For CIP8, a ubiquitin ligase activity has been demonstrated [32] and the same might be true for a subgroup of the cluster, which has a high structural similarity with CIP8 (C.H., unpublished data). Thus, this cluster might represent proteins that are functionally redundant to some extent. Notably, the similarity among members of cluster 2.2 also extends to their genomic organization: 22 of the 26 proteins are encoded by a single exon, underpinning their close relatedness and probable common evolutionary origin.

Conclusions

Gene redundancy in *Arabidopsis* has previously been shown to limit the number of mutants detectable by phenotype [33]. The completed genome sequence shows that a high degree of redundancy might indeed obscure the quest for many phenotypes. Accordingly, we suggest that there probably exists a high degree of functional redundancy among *Arabidopsis* RING-domain proteins. This would also correlate with the fact that surprisingly few genes in the complete set are characterized as mutants. To our knowledge, this is the case for only two of them, COP1 and PRT1 [34,35]. Notably, for both these proteins, a functional requirement for the RING domain has been demonstrated, and both are unique with respect to their RING domains.

In this study, we present an ordered set of manually curated RING domains of *Arabidopsis*. In summary, our set includes all *bona fide* RING domains, as well as common RING-variant domains. Notably, additional *Arabidopsis* proteins might have potential to form variant RING-finger domains, as has been suggested, for instance, for the HOS1 protein [36]. However, their primary sequences do not support this notion unambiguously and we chose not to

Table 3

List of Arabidopsis RING domains

	Protein-ID	S1	S2	S3	S4	Alternative names and remarks
(I) RING-HC: 118 domains in 111 proteins						
Cluster 1.1	At1g19310	+	+	+	+	
	At1g74990	+	+	+	+	
	At2g23780	+	+	+	+	
	At2g42030	+	+	+	+	
	AT3g58030	+	+	+	+	
	AT4g03510	+	+	+	+	RMA1, ubiquitin ligase activity [29]
	AT4g27470 AT4g28270	+	+	+	+	
Cluster 1.2	At1g62370	+	+	-	-	
	AT3g07120	+	+	-	-	
	AT3g25030	+	+	-	-	
	AT4g03960	+	+	-	-	
	AT4g13100	+	+	-	-	
	AT4g22250	+	+	-	-	
Cluster 1.3	At1g57800	+	+	+	+	Second motif in protein
	At1g57820	+	+	+	+	Second motif in protein
	At1g66040	+	+	+	+	Second motif in protein
	At1g66050	+	+	+	+	Second motif in protein
	AT4g08590	+	+	+	+	
	AT5g39550	+	+	+	+	Second motif in protein
Cluster 1.4	At1g57800	+	+	+	-	Second motif in protein
	At1g57820	+	+	+	-	Second motif in protein
	At1g66040	+	+	+	-	Second motif in protein
	At1g66050	+	+	+	-	Second motif in protein
	AT5g39550	+	+	+	-	Second motif in protein
Cluster 1.5	AT3g06140	+	+	+	+	
	AT3g09770	+	+	+	+	
	AT3g53410	+	+	+	+	
	AT5g03200	+	+	+	+	
	AT5g19080	+	+	+	+	
Cluster 1.6	At1g24440	+	+	+	+	
	AT3g47160	+	+	+	+	Potential RNA-binding protein
	AT5g01520	+	+	+	+	
	AT5g58790	+	+	+	+	
Cluster 1.7	At1g66620	+	-	-	-	
	At1g66630	-	-	-	-	Likely not to be a RING
	AT5g37870	+	-	-	-	
	AT5g37910	+	-	-	-	
Cluster 1.8	At2g41980	+	-	-	-	
	AT3g58040	+	-	-	-	
	AT3g61790	+	-	-	-	Seven in absentia-like
	AT4g27880	+	-	-	-	
Cluster 1.9	At1g69330	+	+	+	-	
	At1g74370	+	+	+	-	
	AT3g29270	+	+	+	-	
Cluster 1.10	At1g01350	+	+	+	+	
	AT4g01020	+	+	+	+	Second motif in protein
	AT5g06420	+	+	+	+	

Table 3 (continued)

List of Arabidopsis RING domains

	Protein-ID	S1	S2	S3	S4	Alternative names and remarks
Cluster 1.11	At1g30860	+	+	+	+	
	At2g34920	+	+	+	+	
	AT5g44690	+	+	+	-	
Cluster 1.12	AT5g07270	+	+	+	-	
	AT5g57740	+	+	+	-	
Cluster 1.13	At1g59560	+	-	-	-	
	At1g63900	+	-	-	-	
Cluster 1.14	At1g79110	+	-	-	-	
	AT3g12920	+	-	-	-	
Cluster 1.15	AT5g22750	+	+	+	+	
	AT5g43530	+	+	+	+	Putative DNA-repair protein
Cluster 1.16	At1g03370	+	-	-	-	
	AT4g03000	+	-	-	-	
Cluster 1.17	AT3g01650	+	-	-	-	
	AT5g14420	+	-	-	-	
Cluster 1.18	At1g03770	+	+	+	+	
	AT5g44280	+	+	+	+	
Cluster 1.19	At1g32530	+	+	-	-	
	At2g35330	+	+	-	-	
Cluster 1.20	At1g10650	+	-	-	-	Apoptosis inhibitor-like
	At1g60610	+	-	-	-	
Cluster 1.21	At2g38190	+	-	-	-	
	AT5g01450	+	-	-	-	
Cluster 1.22	At2g47090	+	+	+	+	
	AT3g62240	+	+	+	+	
Cluster 1.23	AT4g19700	+	-	-	-	
	AT5g45100	+	-	-	-	
Cluster 1.24	AT3g23280	-	-	-	-	Likely not to be a RING
	AT4g14360	+	-	-	-	Ankyrin-like protein
Unique	At1g05050	+	-	-	-	Putative transcription factor
	At1g05120	+	+	+	-	
	At1g18660	+	+	+	+	
Cluster 1.25	At1g21650	+	+	+	-	Putative SecA-type plastid protein transport factor
	At1g32740	+	-	-	-	
	At1g55250	+	+	+	+	
	At1g61620	+	+	+	+	
	At1g67180	+	+	+	+	
	At1g67800	+	-	-	-	
	At1g68820	+	-	-	-	
	At1g79380	+	-	-	-	
	At1g79810	-	-	-	-	Likely not to be a RING
	At2g22010	+	+	+	+	
At2g25380	+	+	+	+		
At2g26350	+	+	+	+	Putative peroxisome membrane protein	

Table 3 (continued)**List of *Arabidopsis* RING domains**

Protein-ID	S1	S2	S3	S4	Alternative names and remarks
At2g28840	+	+	-	-	Ankyrin repeat protein
At2g30580	+	+	+	+	
At2g32950	+	+	+	+	COPI, putative ubiquitin ligase [39]
At2g39100	+	+	+	+	
At2g40770	+	+	+	-	SNF2/SWI2-like protein
At2g44410	+	+	+	+	
At2g44950	+	+	+	+	
AT3g05250	+	+	+	-	
AT3g05670	+	+	+	+	
AT3g07200	+	+	+	-	
AT3g24800	+	+	+	+	PRT1, N-end rule ubiquitin ligase [35] Second motif in protein
AT3g24800	+	+	+	-	PRT1, N-end rule ubiquitin ligase [35] Second motif in protein
AT3g26730	+	+	+	+	
AT3g27330	+	+	+	+	
AT3g54360	+	+	+	+	
AT4g01740	+	+	+	+	
AT4g17680	+	+	+	+	
AT4g21070	+	+	+	-	
AT4g33940	+	+	+	+	
AT5g01160	+	+	+	+	
AT5g05130	+	+	+	+	Helicase-like protein
AT5g13530	+	+	+	-	Ankyrin repeat protein
AT5g19430	+	+	+	+	
AT5g23110	+	+	-	-	
AT5g47050	+	-	-	-	
AT5g63700	+	+	+	+	
(2) RING-H2: 215 domains in 214 proteins					
Cluster 2.1	At1g04360	+	+	+	-
	At1g20810	+	+	+	+
	At1g22500	+	+	+	+
	At1g23980	+	+	+	+
	At1g28040	+	+	+	+
	At1g32360	+	+	+	+
	At1g33480	+	+	+	+
	At1g35330	+	+	+	+
	At1g49200	+	+	+	+
	At1g49210	+	+	+	+
	At1g49220	+	+	+	+
	At1g49230	+	+	+	+
	At1g53820	+	+	+	+
	At1g72200	+	+	+	+
	At1g72220	+	+	+	+
	At1g72310	+	+	+	+
	At1g74410	+	+	+	+
	At1g76410	+	+	+	+
	At2g17460	+	+	+	+
	At2g17730	+	+	+	-
	At2g18650	+	+	+	+
	At2g20030	+	+	+	-
	At2g27940	+	+	+	-
	At2g34990	+	+	+	+
	At2g35000	+	+	+	+
	At2g35420	+	+	+	+
					ATL3
					Pseudogene

Table 3 (continued)**List of *Arabidopsis* RING domains**

Protein-ID	S1	S2	S3	S4	Alternative names and remarks
At2g35910	+	+	+	+	
At2g37580	+	+	+	+	
At2g42350	+	+	+	+	
At2g42360	+	+	+	+	
At2g46160	+	+	+	+	
At2g47560	+	+	+	+	
AT3g03550	+	+	+	+	
AT3g05200	+	+	+	-	ATL6
AT3g10910	+	+	+	+	
AT3g11110	+	+	+	+	
AT3g14320	+	+	+	+	
AT3g16720	+	+	+	+	
AT3g18930	+	+	+	+	
AT3g19140	-	-	-	-	Likely not to be a RING
AT3g48030	+	+	+	+	
AT3g60220	+	+	+	+	ATL4
AT3g61550	+	+	+	+	
AT3g62690	+	+	+	+	ATL5
AT4g09100	+	+	+	+	
AT4g09110	+	+	+	+	
AT4g09120	+	+	+	+	
AT4g09130	+	+	+	+	
AT4g10150	+	+	+	+	
AT4g10160	+	+	+	+	
AT4g15970	+	+	+	+	
AT4g17910	+	+	+	+	
AT4g17920	+	+	+	+	
AT4g28890	+	+	+	-	
AT4g30400	+	+	+	+	
AT4g35480	+	+	+	+	RHA3b
AT4g35840	+	+	+	-	
AT4g38140	+	+	+	-	
AT4g40070	+	+	+	-	
AT5g01880	+	+	+	+	
AT5g05280	+	+	+	+	
AT5g05810	+	+	+	+	
AT5g06490	+	+	+	+	
AT5g07040	+	+	+	-	
AT5g10380	+	+	+	+	
AT5g17600	+	+	+	+	
AT5g27420	+	+	+	-	
AT5g40250	+	+	+	+	
AT5g42200	+	+	+	+	
AT5g43420	+	+	+	-	
AT5g46650	+	+	+	+	
AT5g47610	+	+	+	+	
AT5g57750	+	+	+	+	
AT5g58580	+	+	+	+	
AT5g66070	+	+	+	-	
Cluster 2.2	At1g14200	+	+	+	-
	At1g26800	+	+	+	+
	At1g55530	+	+	+	+
	At1g60360	+	+	+	+
	At1g68180	+	+	+	+
	At2g03000	+	+	+	+
	At2g39720	+	+	+	+
	At2g40830	+	+	+	+
	At2g44330	+	+	+	+
	AT3g02340	+	+	+	+
	AT3g10810	+	+	+	+

Table 3 (continued)

List of Arabidopsis RING domains

	Protein-ID	S1	S2	S3	S4	Alternative names and remarks
	AT3g13430	+	+	+	+	
	AT3g19950	+	+	+	+	
	AT3g30460	+	+	+	+	
	AT3g46620	+	+	+	+	
	AT3g60080	+	+	+	+	
	AT4g26400	+	+	+	+	
	AT5g01980	+	+	+	+	
	AT5g02750	+	+	+	+	
	AT5g08140	+	+	+	+	
	AT5g15820	+	+	+	+	
	AT5g20910	+	+	+	+	AIP2
	AT5g56340	+	+	+	+	
	AT5g59550	+	+	+	+	
	AT5g60820	+	+	+	+	
	AT5g64920	+	+	+	+	CIP8, ubiquitin ligase activity [32]
Cluster 2.3	At1g17970	+	+	+	+	
	At1g45180	+	+	+	+	
	At1g53190	+	+	+	+	
	At1g73760	+	+	+	+	
	At2g15530	+	+	+	+	
	AT3g15070	+	+	+	+	
	AT4g31450	+	+	+	+	
	AT4g34040	+	+	+	+	
	AT5g10650	+	+	+	+	
	AT5g24870	+	+	+	+	
	AT5g42940	+	+	+	+	
Cluster 2.4	At1g12760	+	+	+	-	
	At1g63170	+	+	+	-	
	At1g68070	+	+	+	-	
	At1g80400	+	+	+	-	
	AT3g61180	+	+	+	-	
	AT4g11680	+	+	+	+	
	AT4g26580	+	+	+	+	
	AT4g32600	+	+	+	-	
Cluster 2.5	At1g63840	+	+	+	-	
	AT3g43430	+	+	+	+	
	AT3g61460	+	+	+	+	
	AT4g11360	+	+	+	-	RHA1b
	AT4g11370	+	+	+	-	
	AT5g20880	+	+	+	+	
	AT5g41400	+	+	+	-	
Cluster 2.6	AT5g37200	+	+	+	+	
	AT5g37230	+	+	+	+	
	AT5g37250	+	+	+	+	
	AT5g37270	+	+	+	+	
	AT5g37280	+	+	+	+	
Cluster 2.7	AT3g02290	+	+	+	+	
	AT4g23450	+	+	+	+	
	AT5g15790	+	+	+	+	
	AT5g38890	+	+	+	+	
	AT5g41350	+	+	+	+	
Cluster 2.8	AT4g05350	+	+	+	+	
	AT4g12140	+	+	+	+	
	AT4g12150	-	-	-	-	Variant, ligand missing!
	AT4g12190	+	+	+	+	
	AT4g12210	+	+	+	+	

Table 3 (continued)

List of Arabidopsis RING domains

	Protein-ID	S1	S2	S3	S4	Alternative names and remarks
Cluster 2.9	At1g19680	+	+	+	+	
	At1g75400	+	+	+	+	
	At2g21500	+	+	+	+	
	AT4g39140	+	+	+	+	
	AT5g18260	+	+	+	+	
Cluster 2.10	At1g22670	+	+	+	+	
	At1g35630	+	+	+	+	
	At1g71980	+	+	+	+	RMRJR702
	AT4g09560	+	+	+	+	
	AT5g66160	+	+	+	+	RMRJR700
Cluster 2.11	At1g51930	+	+	+	+	
	AT5g41430	+	+	+	+	
	AT5g41440	+	+	+	+	
	AT5g41450	+	+	+	-	
Cluster 2.12	AT3g62970	-	-	-	-	
	AT5g18650	-	-	-	-	
	AT5g22920	-	-	-	-	PGPD14-like protein
	AT5g25560	-	-	-	-	
Cluster 2.13	At1g08050	+	+	+	+	
	At2g38970	+	+	+	+	Putative pol polyprotein
	AT3g54780	+	+	+	+	
	AT5g60710	+	+	+	+	
Cluster 2.14	At1g18910	-	-	-	-	Likely not to be a RING
	At1g74760	-	-	-	-	Likely not to be a RING
	AT3g18290	-	-	-	-	Likely not to be a RING
Cluster 2.15	At2g24480	+	+	+	+	
	AT3g28620	+	+	+	+	
	AT5g43200	+	+	+	+	
Cluster 2.16	At1g15100	-	-	-	-	Likely not to be a RING
	At2g01150	-	-	-	-	RHA2b, likely not to be a RING
Cluster 2.17	At1g65040	+	+	+	+	
	AT3g16090	+	+	+	+	
Cluster 2.18	AT3g14970	+	-	-	-	
	AT3g15740	+	-	-	-	
Cluster 2.19	At2g44580	+	+	+	+	Second nearly identical motif in protein
	At2g44580	+	+	+	+	Second nearly identical motif in protein
Cluster 2.20	At2g22680	+	+	+	-	Putative pol polyprotein
	AT4g37890	+	+	+	+	
Cluster 2.21	At2g18670	+	+	+	+	
	AT4g30370	+	+	+	+	
Cluster 2.22	AT4g25230	-	-	-	-	Likely not to be a RING
	AT5g51450	-	-	-	-	Likely not to be a RING
Unique	At1g04790	+	+	+	+	
	At1g24580	+	+	+	+	

comment

reviews

reports

deposited research

refereed research

interactions

information

Table 3 (continued)**List of *Arabidopsis* RING domains**

Protein-ID	S1	S2	S3	S4	Alternative names and remarks
At1g27010	+	+	+	+	RHY1a
At1g49850	+	+	+	+	
At1g53010	+	+	+	-	
At1g55410	-	-	-	-	Likely not to be a RING
At1g57730	+	+	+	+	
At1g70910	+	+	+	+	RHF1a
At1g74620	+	+	+	+	
At2g04240	+	+	+	-	
At2g05170	+	+	+	+	
At2g15260	+	+	+	-	
At2g15580	+	+	+	-	
At2g20650	+	+	+	-	
At2g26000	+	+	+	+	
At2g28920	+	+	+	-	
At2g47700	+	+	+	-	
AT3g05870	+	+	+	-	
AT3g47180	+	+	+	+	
AT3g47990	+	+	+	-	
AT3g55530	+	+	+	+	
AT3g58720	+	+	+	+	
AT4g01270	+	+	+	+	
AT4g13490	+	+	+	-	
AT4g14220	+	+	+	+	
AT4g18110	+	+	+	+	
AT5g05530	+	+	+	+	
AT5g05910	+	+	+	+	
AT5g54990	+	+	+	+	
AT5g57820	+	+	+	+	
AT5g67120	+	+	+	+	

RING variants

(3) RING-HC, C6 or C7 position substituted or missing: 41 domains in 39 proteins

Cluster 3.1	At2g26130	-	-	-	-	Second motif in protein
	At2g26130	-	-	-	-	Second motif in protein
	AT3g43180	+	+	+	+	
	AT3g43750	-	-	-	-	
	AT3g45510	-	-	-	-	
	AT3g45540	-	-	-	-	
	AT3g45560	-	-	-	-	
	AT3g45570	-	-	-	-	
	AT3g45580	-	-	-	-	
	AT5g60250	+	+	+	+	Second motif in protein
Cluster 3.2	At1g05890	-	-	-	-	
	At1g65430	-	-	-	-	
	At2g31510	-	-	-	-	Dm Ariadne-like
	At2g31760	-	-	-	-	Dm Ariadne-like
	At2g31770	-	-	-	-	Dm Ariadne-like
At2g31780	-	-	-	-	Dm Ariadne-like	
Cluster 3.3	At1g50410	+	+	+	+	Putative DNA-binding protein
	AT3g16600	+	+	+	+	Putative DNA-binding protein
	AT3g20010	+	+	+	+	RUSH1-alpha-like protein
Cluster 3.4	At2g16090	-	-	-	-	
	AT3g27710	-	-	-	-	
	AT4g34370	-	-	-	-	

Table 3 (continued)**List of *Arabidopsis* RING domains**

Protein-ID	S1	S2	S3	S4	Alternative names and remarks	
Cluster 3.5	AT5g63750	-	-	-	-	Dm Ariadne-like
	AT5g63760	-	-	-	-	
Cluster 3.6	AT4g01020	-	-	-	-	Second motif in protein
	AT5g10370	-	-	-	-	
Cluster 3.7	At2g25360	-	-	-	-	
	AT3g45470	-	-	-	-	
Unique	At1g11100	-	-	-	-	RUSH1 alpha-like protein
	At1g32340	-	-	-	-	
	At1g61140	-	-	-	-	
	At2g19610	-	-	-	-	
	AT3g14250	-	-	-	-	
	AT3g45480	+	+	+	+	
	AT3g45580	-	-	-	-	
	AT3g53690	-	-	-	-	
	AT3g54460	-	-	-	-	
	AT4g19670	-	-	-	-	
AT4g19670	-	-	-	-		
AT5g07640	-	-	-	-	Second motif in protein	
AT5g60250	-	-	-	-	Second motif in protein	

(4) RING-H2, C7 position substituted or missing: two domains in two proteins

AT3g42830	+	+	+	+	Rbx1-like
AT5g37220	-	-	-	-	

(5) C4 or C5 position substituted or missing: nine domains in nine proteins

Cluster 5.1	At1g18760	-	-	-	-	
	At1g18770	-	-	-	-	
	At1g18780	-	-	-	-	
	At1g21960	-	-	-	-	
	At2g29840	-	-	-	-	
	AT5g53910	-	-	-	-	Likely not to be a RING
	At1g77830	-	-	-	-	RHA1b-like
Unique	At1g36950	-	-	-	-	
	At2g34000	-	-	-	-	

(6) C2 position substituted or missing: two domains in two proteins

At2g37150	-	-	-	-	
AT5g63760	-	-	-	-	Second motif in protein

List of *Arabidopsis* proteins (Protein-ID) that contain a manually curated RING domain according to the criteria described in the text. The table is divided into three principal sections: RING domains of the RING-HC type; RING domains of the RING-H2 type; and RING-variant domains. In addition, the domains are ordered in numbered clusters according to a single-linkage-clustering method with a 10^{-15} threshold as described in the text. If an individual domain meets the requirements of one of the stringent patterns 1 to 4 (S1-S4) (see Table 2), it is indicated by a plus sign, if not, it is indicated by a minus sign. Additional remarks such as previously assigned gene names and significant similarities are given in the last column. The remark 'likely not to be a RING' refers to the presence of an aromatic residue two positions in front of metal-ligand position 7 as outlined in the text.

include any RING-domain variants in our analysis for which no structural experimental evidence is yet available. Clearly, our findings show that predictions of cysteine-rich domains have to be met with skepticism. On a proteomics level, they can be misleading in drawing general conclusions, as is amply demonstrated by the overestimation of the abundance of the PHD domain owing to their overlapping classification with RING domains. Additional structural data are needed and have to be taken into account in computational analyses to resolve these issues. Our curated set of RING domains in *Arabidopsis* will serve as a vital starting point for further genome analysis in this field.

Materials and methods

The non-redundant *Arabidopsis* genome protein set available at MIPS [37] was screened for proteins containing RING-finger domains. Detailed results of this analysis are available on the web [20,21]. Analysis was undertaken using several discrete steps described in detail below.

Whole-genome analysis for proteins containing RING-finger motifs proteins

For initial analysis the InterPro system [2] (iprscan version 3.2) was used to calculate protein domains for all *Arabidopsis* proteins. The results were filtered for RING-finger domains matching the InterPro domains PF00097, PS00518 or PS50089 (corresponding to domain names ZF-C3HC4, ZINC_FINGER_C3HC4 and ZF_RING, respectively). *Arabidopsis* proteins containing one or more RING-finger domains were analyzed further.

Frequently detected overlapping domains, for example detected patterns with overlapping localization, were unified and only the domain with the most amino-terminal starting point was used for further analysis.

Prediction of additional domains

Proteins containing a RING-finger domain were subjected to an additional screen for the presence of additional domains using the InterPro package (see above).

Classification of RING-finger domains

The RING-finger domain summarizes different types of sub-domains, namely the C3HC4-type and C3H2C3-type. We refer to these types as RING-HC and RING-H2, respectively. To differentiate between these two subtypes an additional fine analysis was carried out: RING-finger-containing genes were classified as C3HC4-type (RING-HC) for the patterns C-x-H-x-[LIVMFY]-C-x(2)-C-[LIVMYA] or C-x(2)-C-x(9-39)-C-x(1-3)-H-x(2-3)-C-x(2)-C-x(4-48)-C-x(2)-C and as C3H2C3-type (RING-H2) for the patterns C-x-H-x-[LIVMFY]-H-x(2)-C-[LIVMYA] or C-x(2)-C-x(9-39)-C-x(1-3)-H-x(2-3)-H-x(2)-C-x(4-48)-C-x(2)-C. RING-finger domains detected by InterPro that did not match these patterns were marked as 'others/unclear type'. Novel patterns for the evaluation

of RING domains were defined as described in the text and Table 2.

Clustering of RING-finger domains

The isolated RING domains were related using BLASTP [25] (version 2.1.1.2) by testing the isolated RING-finger domains against a database containing all RING-finger domains assembled during the previous analysis steps. Domains below a threshold of 10^{-15} were united into clusters of related domains. This procedure is 'greedy'; for example, although domain A relates to domain B and B relates to C, A and C are not necessarily closely related enough to exceed the given threshold. Nevertheless, this procedure in general succeeded in grouping and/or separating individual subfamilies.

Multiple alignments

For RING-domain clusters with two or more members, multiple alignments of the respective complete protein sequences were done using the ClustalW program [26,38] with default parameter settings.

Manual expert curation

The individual RING-finger domains and clusters underwent manual inspection. Manual adjustments to clusters and rejections of individual domains and clusters on the basis of expert knowledge were carried out as explained in Results and discussion.

Additional data files

The curated set of clustered *Arabidopsis* RING domains, with their sequences and metal ligands, are provided in a supplemental table with the online version of this article. Links to the individual genes and ClustalW analyses are included.

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