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Review

The RCC1 superfamily: From genes, to function, to disease

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ABSTRACT

The Regulator of Chromosome Condensation 1 (RCC1) was identified over 20 years ago as a critical cell cycle regulator. By analyzing its amino acid sequence, RCC1 was found to consist of seven homologous repeats of 51–68 amino acid residues, which were later shown to adopt a seven-bladed β-propeller fold. Since the initial identification of RCC1, a number of proteins have been discovered that contain one or more RCC1-like domains (RLDs). As we show here, these RCC1 superfamily proteins can be subdivided in five subgroups based on structural criteria. In recent years, a number of studies have been published regarding the functions of RCC1 superfamily proteins. From these studies, the emerging picture is that the RLD is a versatile domain which may perform many different functions, including guanine nucleotide exchange on small GTP-binding proteins, enzyme inhibition or interaction with proteins and lipids. Here, we review the available structural and functional data on RCC1 superfamily members, paying special attention to the human proteins and their involvement in disease.

1. Introduction

The RCC1 superfamily of proteins is characterized by a 350–500 residue domain, known as the RCC1-like domain or RLD, that was first reported in 1987 in the Regulator of Chromosome Condensation 1, RCC1 [1,2]. A mutated RCC1 allele was found to be responsible for the temperature-sensitive phenotypes seen in the ts-BN2 hamster cell lines, including premature chromosome condensation and arrest in the G1 phase of the cell cycle. These phenotypes could be rescued by the introduction of wild-type RCC1 [1–3]. It was not until the mid-nineties that another human protein, HERC1, was found that contained not one, but two RLDs [4]. Since then, sixteen additional human proteins have been described that have at least one RLD in their amino acid sequences.

In this review, we show that these proteins can be divided into five subgroups based on their structural characteristics (Fig. 1): (1) the RCC1 subgroup, (2) the HERC subgroup, (3) the RCBTB subgroup, (4) the kinase subgroup and (5) the miscellaneous subgroup, encompassing the proteins that cannot be classified into any of the other subgroups.

In addition, the following sections will present the available knowledge regarding the structure and function of RCC1 superfamily genes and proteins, with an emphasis on the human members. Finally, the involvement of RCC1 superfamily genes and proteins in pathological conditions will also be discussed.

2. RCC1 superfamily genes and proteins

The genes encoding human RLD-containing proteins (see Table 1) are located in different chromosomes, though closer homologs tend to be located in the vicinity of each other. Thus, the genes for RCC1 and TD-60 are close to each other in chromosome 1. Similarly, HERC1 and HERC2 are both on the long arm of chromosome 15, whereas HERC3, HERC5 and HERC6 are in the same region in chromosome 4 [5]. Likewise, both RCBTB1 and RCBTB2 are located on the long arm of chromosome 13. The other genes are dispersed throughout the genome, either on autosomes or, in the case of RPGR, in the X chromosome.

All these genes have different exon numbers and the proteins encoded by them range from little more than 400 amino acid residues to close to 5000 (see Table 1). Splicing isoforms have been studied in some cases, such as those of RCC1, DelGEF, RPGR, PAM and some HERC proteins [6–10]. While alternative splicing of RCC1, HERC6 and PAM does not affect the sequence of their RLDs [6,7,10], the same cannot be said of DelGEF, RPGR and HERC4, where some of the isoforms have truncated RLDs [7,8,11,12]. Apart from that, the Ensembl database also predicts alternatively spliced transcripts for some of the other proteins described here, but whether or not these isoforms actually exist has not yet been examined [13].

Regarding the architectures of RCC1 superfamily proteins (Fig. 1), there are instances where the RLD domain makes up almost the whole protein, as is the case for RCC1 subgroup members (RCC1, TD-60, DelGEF and WBSCR16), or it can be the only highly conserved region of a protein, as in RPGR. In other cases, the RLD is part of more complex, multidomain proteins. For example, RLDs can be found in proteins involved in ubiquitination, where they are associated with HECT or

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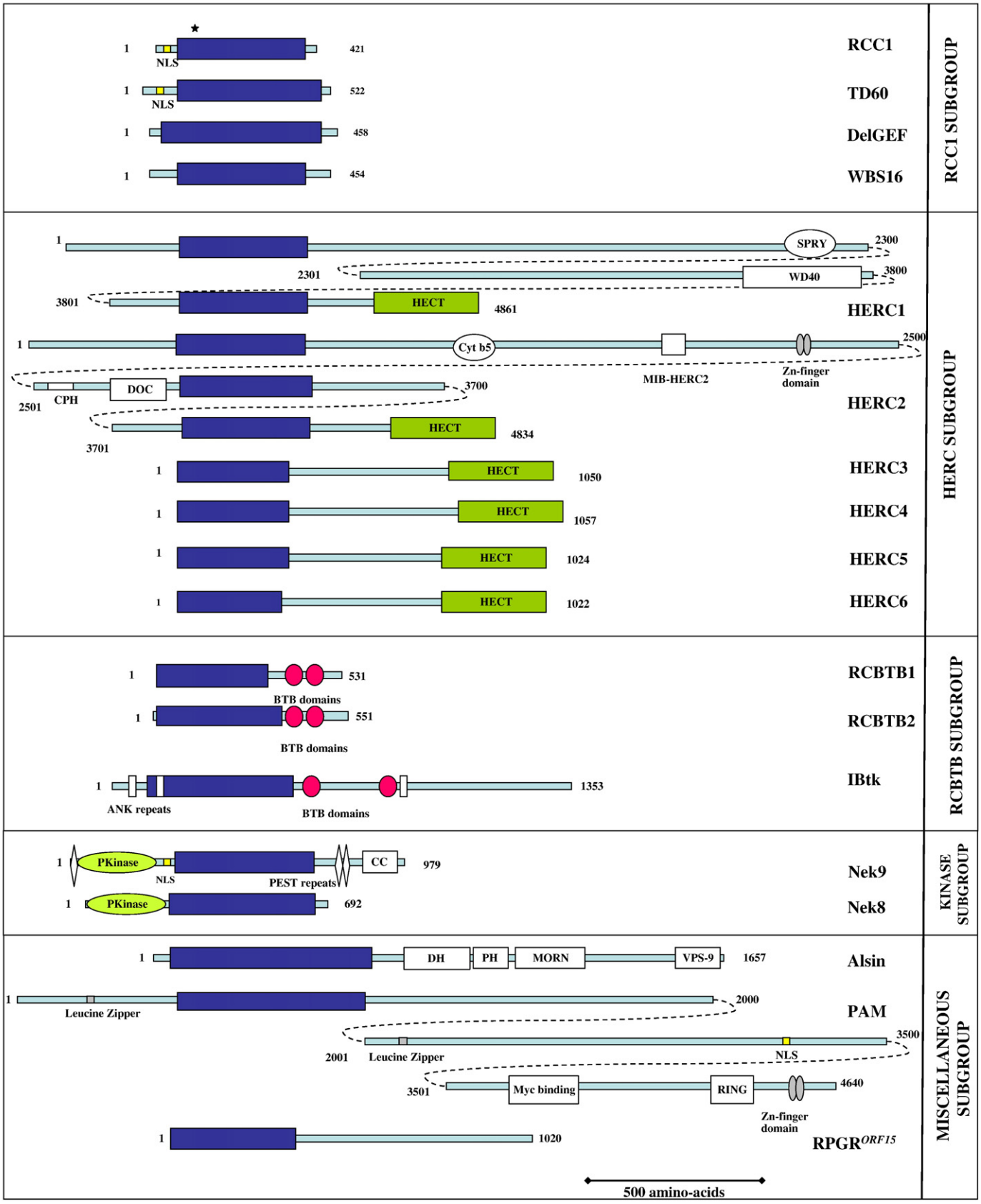


Fig. 1. Schematic representation of human RCC1 superfamily proteins. The eighteen proteins have been classified into five subgroups: (1) the RCC1 subgroup (including those proteins whose RLD spans almost the whole length of the protein), (2) the HERC subgroup (proteins containing RLD and HECT domains), (3) the RCBTB subgroup (proteins containing RLD and BTB domains), (4) the kinase subgroup (proteins containing RLD and kinase domains) and (5) the miscellaneous subgroup, encompassing those proteins that do not fit into any of the previous categories. Dark blue rectangles represent the RLDs. All proteins and domains are shown to scale (scale shown at bottom right side). Big proteins such as PAM, HERC1 and HERC2 are drawn in more than one line, with amino acid numbers shown at each end. A star on top of RCC1's RLD shows the location of the β-wedge, essential for RCC1's activity as a Ran-GEF.

Table 1
The human RCC1 superfamily proteins

The human RCC1 superfamily								
	Protein names	Number of residues	Protein/gene accession number	Chromosomal location	Known function/interaction	RLD function	Other known domains	References
RCC1 subgroup	RCC1 (Regulator of Chromosome Condensation 1), Ran-GEF (RLD: 18–421)	421	P18754/ NM_001269	1p36.1	Binds to chromatin and acts as a guanine exchange factor (GEF) on Ras homologue Ran	G,P	Nuclear localization signal (NLS)	[1–3,15,22, 29,31,66]
	TD-60 (telophase disk 60), RCC2 (RLD: 121–507)	522	Q9P258/ NM_018715	1p36.13	Chromosomal passenger protein involved in mitotic progression	?	NLS	[28,30,77]
	DelGEF (deafness locus-associated putative guanine nucleotide exchange factor), also called SERGEF. (RLD: 4–407)	458	Q9UGK8/ NM_012139	1p14.3	Modulates secretion of proteoglycans and interacts with Sec5 and DelGIP1	?		[8,32,33]
	WBSCR16 (William–Beuren syndrome critical region 16), WBS16 (RLD: 56–454)	454	Q8N572/ NM_030798	7q11.23	Possible role in Williams–Beuren syndrome	?		[152]
HERC subgroup	HERC1 (for HECT domain and RCC1) (RLD1: 361–749; RLD2: 3990–4366)	4861	Q15751/ NM_003922	15q22	Binds clathrin heavy chain, phosphoinositides, ARF/Rab proteins and TSC2. Ubiquitin ligase, no known targets	G,P,L	HECT, SPRY, WD40, leucine zipper	[4,5,42–44, 79,80]
	HERC2 (RLD1: 415–784; RLD2: 2949–3332; RLD3: 3941–4324)	4834	O95714/ NM_004667	15q13	Involved in murine rjs syndrome. ubiquitin ligase, no known targets	?	HECT, Mib-HERC2, DOC domain, ZZ type zinc finger, cyt *b*5-like region	[5,34, 141–148]
	HERC3 (RLD: 1–371)	1050	Q15034/ NM_014606	4q21	Ubiquitin ligase, no known targets	?	HECT	[5,35,41]
	HERC4 (RLD: 1–371)	1057	Q5GLZ8/ NM_015601 or NM_022079	10q21.3	Ubiquitin ligase, no known targets	?	HECT	[7,45]
	HERC5 (previously known as Ceb1) (RLD: 1–367)	1024	Q9UII4/ NM_016323	4q22.1	Mediates ISGylation of natural ISG15 target proteins	?	HECT	[7,36–40]
	HERC6 (RLD: 1–355)	1022	Q8IVU3/ NM_017912	4q22.1	Ubiquitin ligase, no known targets	?	HECT	[7,37–40]
RCBTB subgroup	RCBTB1 (RCC1 and BTB containing protein 1) or CLLD7 (RLD: 1–357)	531	Q8NDN9/ NM_018191	13q14	Possible tumor suppressor in chronic lymphocytic leukemia	?	BTB domain	[154–156]
	RCBTB2, also called CHC1L (RLD: 1–372)	551	O95199/ NM_001268	13q14.3	Possible tumor suppressor in prostate cancer	?	BTB domain, BRCA domain	[157]
	IBtk (inhibitor of Bruton's tyrosine kinase) (RLD: 74–485)	1353	Q9P2D0/ NM_015525	6q14.1	Inhibits Btk	?	BTB, ANK	[52]
Kinase subgroup	Nek9, also known as Nercc1 (RLD: 332–725)	979	Q8TD19/ NM_033116	14q24.3	Role in mitotic progression. Binds to Ran.	P	Protein kinase, CC, PEST sequences	[46–49, 81–83]
	Nek8 (RLD: 272–683)	692	Q86SG6/ NM_178170	17q11.1	Role in ciliary size and function	?	Protein kinase	[46,50,51, 84–86, 135–140]
Miscellaneous subgroup	Alsin also called ALS2 (RLD: 110–633)	1657	Q96Q42/ NM_020919	2q33.1	Rab5-GEF and Rac1 effector, though not through RLD domain, binds to GRIP1	G, P	DH, PH, MORN, VPS9	[57–59,87–89, 125,126,134]
	PAM (protein associated with Myc) also called Myc binding protein 2 (MYCB2) (RLD: 552–1030)	4640	O75592/ NM_015057	13q22	Binds to Myc and inhibits adenylyl cyclase	P	RZF, 2 Zinc-fingers, Myc binding protein, NLS	[10,60–63, 95–117]
	RPGR (retinitis pigmentosa GTPase regulator), ORF15 (RLD: 1–372)	1020	Q92834/ NM_000328	Xp11.4	Role in ciliary function. Linked to retinitis pigmentosa, binds to PDEδ and RPGRIP1	P		[9,11,12, 53–56,90–94]

In the RLD function column, G, P and L stand for: guanine nucleotide exchange/release factor (G), protein–protein interactions (P), and protein–lipid interactions (L). A question mark indicates that no functions have been identified for that RLD.

RING finger domains (the HERC proteins and Pam), or in proteins with other catalytic activities, such as kinases. RLDs can also be associated with protein–protein interaction domains such as the BTB domain (Broad complex, Tramtrack and Bric à brac) of the RCBTB subgroup.

Concerning the RLDs themselves, these are domains of usually around 400 amino acid residues (from 355 to 411), with the exceptions of PAM and Alsin, whose RLDs are bigger than the rest at 478 and 523 residues, respectively (Table 1). Fig. 2 shows an alignment of all reported human RLDs, i.e. those present in the proteins shown in Fig. 1. The RLDs shown in these two figures are recognized as such by the InterPro database (entry IPR009091) [14] and also by the Superfamily database (entry 50985) (http://supfam.cs.bris.ac.uk/SUPERFAMILY). In

the former webpage, information is available on the hidden Markov model-based algorithm used for establishing the presence and location of RLDs in protein sequences.

As previously described, the RLD of RCC1 is made of seven repetitive sequences of 51–68 amino acid residues each, which together constitute the full domain [15]. These repeats, known as RCC1 repeats, are defined by another algorithm that can be found in the Prosite database (entry PS50012) [16], or in InterPro (entry IPR000408) [14]. Although all RLD sequences align well with the RLD of RCC1 (Fig. 2), not all of them contain seven RCC1 repeats according to the definition just mentioned. Thus, while the RLDs of RCC1, DelGEF, HERC1 (both RLDs), HERC2 (the last two RLDs), HERC3 and HERC4 all contain seven canonical RCC1 repeats, all other RLDs contain between three and six RCC1 repeats (PAM and IBtk have three; RCBTB2, HERC5 and the first RLD in HERC2 have four; TD-60, HERC6, RCBTB1, Nek8 and Alsin have five; WBSCR16, Nek9 and RPGR have six). However, the absence of seven canonical RCC1 repeats in these RLDs should not be interpreted as meaning that these RLDs do not have seven repeats. As a matter of fact, all these RLDs have sequences that correspond, both in length and in similarity (Fig. 2), to each of the seven repeats in RCC1. Therefore, these RLDs do indeed contain seven repeats, some of which qualify as bona fide RCC1 repeats, while the rest are non-canonical RCC1-like repeats [17]. The next section will address the relationship between these sequence repeats and the three-dimensional structure of these proteins.

In Fig. 3A, a phylogenetic tree of all known human RLDs is shown. As explained in the figure legend, this tree was generated from the alignment in Fig. 2. Interestingly, the tree shows that the five subgroup-based classification mentioned earlier for RLD-containing proteins (Fig. 1) does not accurately reflect the phylogenetic relationships between RLDs. Thus, for the RCC1 subgroup, the RLDs of RCC1, TD-60 and WBSCR16 are closely related, but they are very different from that of DelGEF. Regarding the HERC subgroup, their RLDs cluster in two separate regions, corresponding to the large (HERC1–2) and small (HERC3–6) HERC proteins, respectively. In the RCBTB subgroup, all three RLDs are closely related to each other, as happens with the Kinase subgroup. Finally, the miscellaneous subgroup has no phylogenetic basis at all, since its three members are not closely related to each other: Alsin RLD is most similar to the RLDs of small HERCs, PAM's RLD is closest to those in the RCBTB subgroup and the RLD of RPGR clusters with those of RCC1 subgroup members RCC1, TD-60 and WBSCR16.

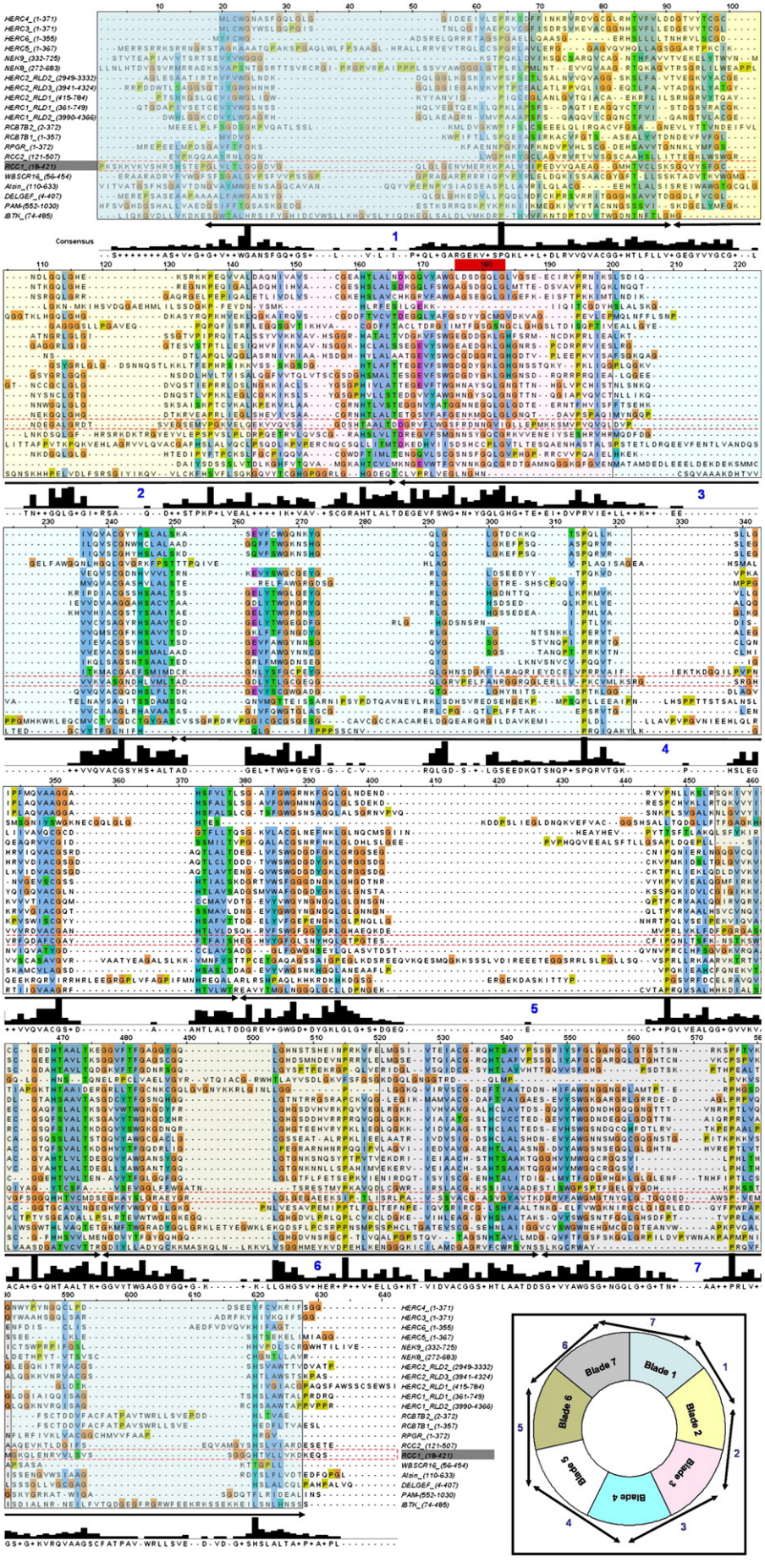
Very little is known regarding the evolution of RLDs. The fact that RCC1 repeats and RLDs are found in all sorts of organisms, including prokaryotes, unicellular eukaryotes, plants, fungi and animals (as shown by the Pfam database, entry PF00415 [18,19]), suggests a very ancient origin for this domain, possibly previous to the appearance of eukaryotic organisms. Nevertheless, other possibilities have been suggested, such that prokaryotes got their RLDs later on by horizontal gene transfer from eukaryotes [17,20]. Even less can be said about the mechanisms that gave rise to the first RLDs. In this respect, the fact that exons in RCC1 roughly match its sequence repeats seems to indicate that the RLD arose by sequential duplication of an ancestral repeat module, but this has yet to be proven [17]. In any case, regardless of how RLDs first came into being, they have proven to be very useful and versatile protein domains, as evidenced by their widespread distribution and their conservation between species.

3. Three-dimensional structure

To this day, only two three-dimensional structures of RLDs have been published [15,21,22]. One of them corresponds to the bacterial β-lactamase inhibitor protein-II (BLIP-II) from *Streptomyces exfoliatus* [21], while the other is that of human RCC1 [15]. Since both structures are remarkably similar, we will focus our discussion mainly on the latter. Ten years ago, Renault et al. reported that the crystal structure of RCC1 resembles that of the WD40 domain, which is present, among others, in the beta subunits of trimeric G proteins [15]. Despite their distinct amino acid sequences, RLD and WD40 domains share a tertiary structure which is reminiscent of a seven-bladed propeller [15]. In RCC1, each of these blades consists of a four-stranded antiparallel β-sheet, with the inner strand of each blade aligned with the central shaft of the propeller, which is filled with water molecules [15]. Interestingly, the seven repeats identified in RCC1 by primary sequence analysis (see above) do not correspond with the blades of the propeller, but rather each sequence repeat contains the third and fourth β-strands of one blade, followed by the first and second β-strands of the next blade [15] (inset Fig. 2). As a result of this, one of the blades in the propeller consists of the last half of the seventh sequence repeat, followed by the first half of the first sequence repeat, an arrangement that behaves as a molecular clasp, closing the circular structure [15]. A superimposition of all seven blades in RCC1 shows a very remarkable structural similarity in the first three β-strands of each blade, suggesting that these strands are the most essential to maintain the overall propeller structure [15]. By contrast, the fourth strand, the outermost one in each blade, is structurally more variable, and it is likely that these strands play a more important role in the interactions of RCC1 with other molecules. Consistent with this, BLIP-II, which also folds as a seven-bladed β-propeller, only has three β-strands per blade, confirming that the outer β-strand is not required to maintain the core propeller structure [21]. Each of the blades in RCC1 contains several highly conserved residues, including three glycine residues that are required for the sharp turns between the different strands, as well as hydrophobic residues that stabilize the antiparallel β-sheets [15]. In the outer strands, there are also several conserved residues, including an invariable *cis*-proline [15]. Both the N- and the C-terminal tails of RCC1 are located on the same side outside the propeller plane, with the N-terminal tail containing a nuclear localization signal (NLS) as well as phosphorylation and methylation sites [23–26]. While N-terminal methylation is required for binding of RCC1's N-terminal tail to nucleosomal DNA, core nucleosomal histones bind to the propeller itself at the same side where N- and C-termini protrude [23,27]. On the opposite side of the propeller, multiple Ran-binding residues can be found, in addition to those involved in catalyzing nucleotide exchange on this small GTP-binding protein [15]. A critical role in this latter function is carried out by the β-wedge, a rigid β-sheet protruding prominently from blade-3 (residues 146–153), which is directly involved in destabilizing the association between Ran and GDP, thus eliciting GDP release and subsequent GTP uptake by Ran [15]. As expected, no β-wedge is present in BLIP-II [21].

Given this wealth of structural data for RCC1, the question arises as to whether RCC1's structural features are conserved in other RCC1 superfamily proteins, and to what extent. In principle, given the sequence homology between RCC1 and the other RLDs, it would seem

Fig. 2. Multiple alignment of the RLDs of all known human RCC1 superfamily proteins. The sequences used correspond to the entries shown in Table 1. This figure has been generated using ClustalW and Jalview [158–160], then edited manually. Alignments are colored using the Clustal-IX scheme in Jalview (glycines are shown in orange, prolines in lime green, small and hydrophobic amino acids (A, V, L, I, M, F, W) in blue, hydroxyl and amine amino acids (S, T, N, Q) in dark green, negatively charged amino acids (D, E) in magenta, positively charged amino acids (R, K) in red and histidines and tyrosines in dark blue. The consensus sequence and degree of conservation (graph in black) are shown under the alignment [158–160]. A phylogenetic tree was generated from this alignment and is shown in Fig. 3A. The RCC1 sequence has been highlighted with a red rectangle (dashed line), and the sequences corresponding to its seven internal repeats are marked by bidirectional arrows [15]. A red filled rectangle around sequence number 180 represents the location of the RCC1 β-wedge. HERC1 and HERC2, which have more than one RLD, have each of their domains numbered. In the bottom right, a schematic representation depicts the relationship between the seven propeller blades (each in different colors also represented in the alignment) of RCC1 and its seven sequence repeats [15].


HERC4_(1-371)
HERC3_(1-371)
HERC6_(1-355)
HERC5_(1-367)
NEK9_(332-725)
NEK8_(272-683)
HERC2_RLD2_(2949-3332)
HERC2_RLD3_(3941-4324)
HERC2_RLD1_(415-784)
HERC1_RLD1_(361-749)
HERC1_RLD2_(3990-4366)
RCBTB2_(2-372)
RCBTB1_(1-357)
RPGR_(1-372)
RCC2_(121-507)
RCC1_(19-421)
WBSCR16_(56-454)
Alsin_(110-633)
DELGEF_(4-407)
PAM-(552-1030)
IBTK_(74-485)
Consensus
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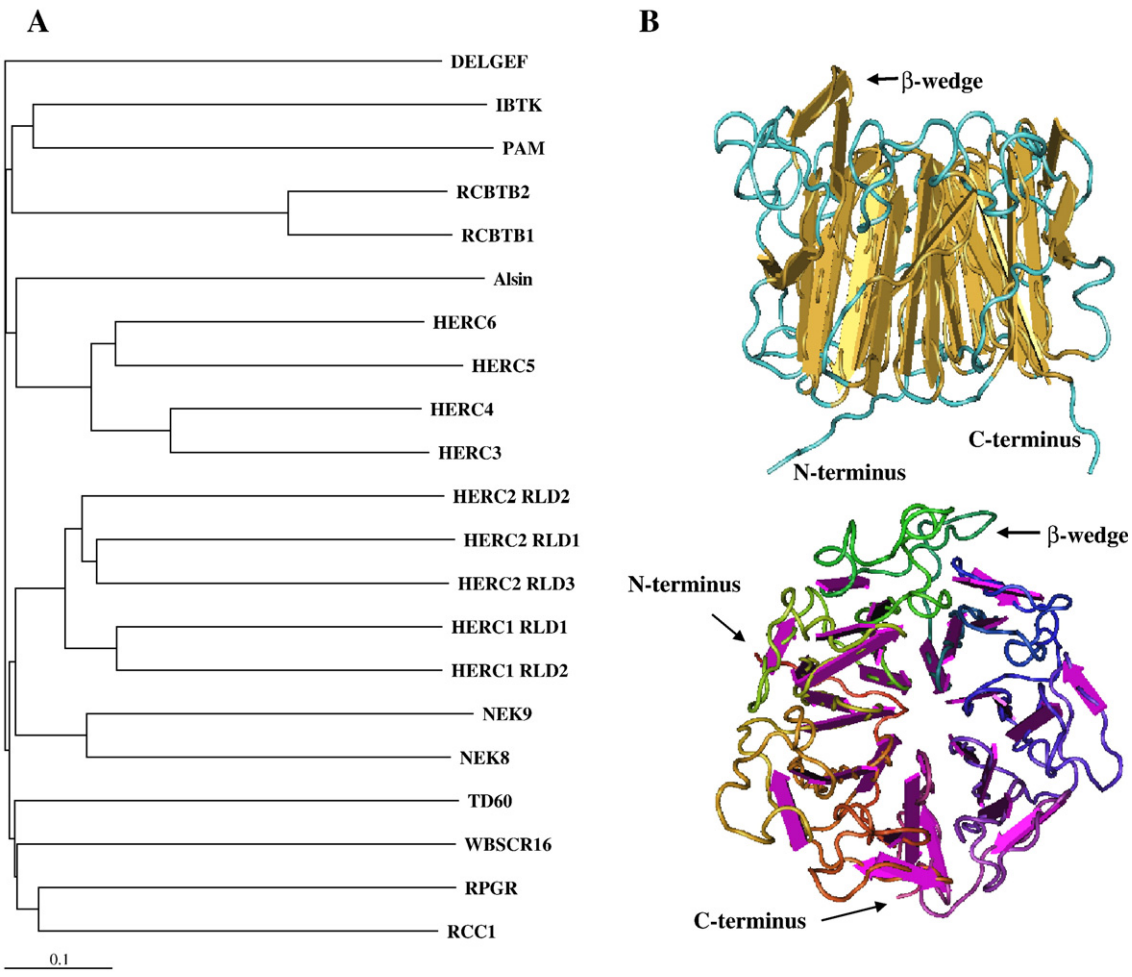

Fig. 3. (A) Phylogenetic tree of the RLDs of all human RCC1 superfamily proteins. The tree was generated using ClustalW and Jalview [158–160]. (B) Human RCC1 1.7 Å structure [15,22]. (top) lateral view of the β-propeller showing the β-wedge, necessary for the GEF activity of RCC1 on Ran, and the N- and C-termini of RCC1. (bottom) frontal view of the β-propeller. The figure was generated and edited using the Cn3D program (www.ncbi.nlm.nih.gov/structure/Cn3D/cn3d.shtml) (Protein Data Bank entry for the human RCC1 structure is 1A12).

likely that many of these RLDs also adopt β-propeller structures. In the following paragraphs, we will present evidence pointing in this direction, but it must be kept in mind that definitive evidence will only be provided by high-resolution structural analyses like those published for RCC1 and BLIP-II [15,21].

A first piece of evidence favouring the hypothesis that most or all RLDs fold as seven-bladed β-propellers lies in the fact that BLIP-II, despite being a bacterial protein and having only four canonical RCC1 repeats (and three non-canonical ones), also adopts this same folding [21], thus indicating that RCC1-like repeats need not be canonical in order to give rise to the antiparallel β-sheets that constitute the blades. In order to further test our hypothesis, we analyzed whether critical residues needed for propeller formation in RCC1 are conserved in the other human RLDs. There are only four residues that are conserved in all seven repeats in RCC1: three glycines needed for the turns between β-strands and a *cis*-proline in the last strand of each blade [15]. We counted how many of these 28 residues in RCC1 were conserved in each of the other RLDs. To do that, we scored as positive hits those cases in which an RLD had the same residue as RCC1 in either the same position (as defined by the alignment in Fig. 2), or up to two residues away from the expected position. Using these criteria, we found that most RLDs (14 out of 20) contain at least 80% of the 28 studied residues in the appropriate positions. For the other six RLDs, the values are over 70% for PAM, Nek8 and Nek9, 60% for IBtk, 50% for Alsin, and only 35% for HERC5. Therefore, on the basis of these data alone, HERC5, Alsin and IBtk would be the least likely candidates to share a common three-dimensional fold with RCC1.

Another hint as to whether other RLDs also fold as seven-bladed β-propellers can be found by looking at the predicted secondary structures for these domains. We used the Hierarchical Neural Network (HNN) secondary structure prediction method (http://npsa-pbil.ibcp.fr) to do this for all the RLDs in Fig. 2. In all cases, these RLDs are predicted to contain multiple β-strands at roughly the expected positions, and these strands are almost invariably followed very closely by glycine residues. This is true even for HERC5, Alsin and IBtk, suggesting that our previous failure to identify these glycines is probably due to these residues not being well aligned in Fig. 2 with their counterparts in the other RLDs. Altogether, the observations presented here suggest that most or all RLDs fold as seven-bladed β-propellers, although, as has been said, definitive proof is not yet available.

Even if the overall propeller structure is broadly conserved among these proteins, there is still room for significant variability among individual RLDs. As mentioned above, the RLD of RCC1 contains an extra β-sheet, also known as the β-wedge, which sticks out of the propeller between the third and fourth β-strands of blade-3. This β-wedge, which is critical for RCC1 function, does not appear to be conserved in other RLDs (Fig. 2, red filled box). The existence of the β-wedge in RCC1 proves an important point, namely that specific structural motifs can be intercalated between the β-strands of the propeller (at least between the third and fourth strands of a blade) without disrupting β-propeller structure, and that these specific motifs may bestow important functions upon the RLDs containing them. Hence, other RLDs may also contain analogous motifs (not necessarily similar to RCC1's β-wedge), which may confer important

and specific activities to these domains without affecting their overall folding pattern. But again, more three-dimensional structures of RLDs will be needed in order to determine whether this is the case or not.

4. Tissue and subcellular localization

While some human RLD-containing proteins, such as RCC1, have essential cellular functions and seem to be expressed in virtually all cell types, others, like IBtk, have more restricted expression patterns and their functions are likely to be more tissue-specific. On the other hand, the subcellular localizations of all these proteins are dependent on which other molecules they interact with inside cells. In the RCC1 subgroup, RCC1 and TD-60, both of which have essential cell cycle functions and are widely expressed, have nuclear localization signals (NLS) [26,28,29] and are therefore located in the nucleus during interphase (it must be noted, however, that TD-60 is only expressed in the G2 and M phases of the cell cycle [30], while RCC1 levels are cell cycle-independent). By contrast, during cell division RCC1 is associated with mitotic chromosomes [24,29], whereas TD-60 is associated with the inner centromeres, from which it migrates to the spindle midzone at later stages of mitosis [28]. While the RLD plays an important role in RCC1's association to chromatin [23,27,31], nothing is known about how TD-60 interacts with centromeres and the mitotic spindle. Likewise, not much is known about the two other RCC1 subgroup members, DelGEF and WBSCR16, other than the fact that DelGEF has two isoforms, one of which (DelGEF1) is located primarily in the nucleus, but also in the cytoplasm, whereas the other (DelGEF2, having a truncated RLD) seems to localize in mitochondria [8,32,33]. Concerning the HERC subgroup, all members are expressed in most if not all tissues, but at varying levels. Thus, HERC1, HERC2, HERC4 and HERC6 mRNAs are especially abundant in brain and testis [4,7,34], HERC3 is more prevalent in brain [7,35] and HERC5 is expressed at much higher levels in testis than in any other tissue [7,36,37]. In addition, HERC5 and HERC6 levels in certain cell types have been shown to be regulated by inflammatory mediators, but this will be dealt with in the following section [37–40]. In terms of their intracellular localizations, these proteins seem to be cytosolic, although they can also be found associated to intracellular membranes by virtue of their interactions with other proteins or lipids [4,5,7,41–45]. Regarding the kinase subgroup, Nek9 appears to be a ubiquitous mitotic regulator, whereas Nek8 mRNA is more abundant in liver, kidney and testes, although it is also present in other tissues [46]. There is some controversy as to whether Nek9 is located mainly in the nucleus or the cytosol [47–49]. In any case, however, it is clear that Nek9 has an NLS and the nuclear-to-cytoplasm ratio of the protein can be affected by the adenoviral E1A protein [49]. On the other hand, Nek8 has been shown to reside in the cytoplasm and in the proximal region of primary cilia in kidney epithelial cells [50,51]. Not much is known about the localization of RCBTB subgroup members, apart from the fact that IBtk seems to be specifically expressed in lymphocytes, where it is found underlying the plasma membrane [52]. As to the miscellaneous subgroup, an interesting case is that of RPGR, for which several isoforms have been reported [9,11,12]. Apart from a recently described isoform which predominates in the inner segments of retinal cones and whose RLD is slightly truncated [11], the two main RPGR isoforms seem to be $RPGR^{ex1-19}$ and $RPGR^{ORF15}$. Both share the same RLD but differ in their C-termini, where $RPGR^{ORF15}$ has a glutamic acid-rich domain and a nucleophosmin-binding domain that are absent in $RPGR^{ex1-19}$ [9,53]. Both isoforms associate with microtubule-containing structures such as the connecting cilia of retinal photoreceptors, where $RPGR^{ORF15}$ predominates [54], or the transitional zone of motile cilia in airway epithelia, where only $RPGR^{ex1-19}$ can be found [54]. In addition, $RPGR^{ORF15}$ localizes to centrosomes/spindle poles in cultured cell lines that lack cilia as well as in the basal body in ciliated cell lines [53]. $RPGR^{ORF15}$ can also be found in the tip and axoneme of sperm flagella [55]. Although RPGR interacts with a number of different proteins, the RLD-mediated interaction of RPGR with RPGRIP is of critical importance to target RPGR to the photoreceptor connecting cilium [56]. Regarding the Alsin protein, its levels have been found to be highest in brain, but other tissues also contain smaller amounts of the protein [57]. Alsin has been localized to multiple sites within the cell: the cytosol, endocytic compartments, including early endosomes and macropinosomes, and actin-rich structures such as membrane ruffles and lamellipodia [57–59]. Interestingly, the RLD of Alsin seems to prevent binding to these membrane-bound compartments, maybe because of an intramolecular interaction between the N-terminal RLD and the C-terminus of the protein [59]. Finally, PAM is expressed ubiquitously but with higher mRNA levels in brain and thymus [60]. In mouse and rat brains, mRNA levels of the respective PAM orthologs are most abundant in the pyramidal cells of the hippocampus and in the granule cells of the dentate gyrus and cerebellum [61]. PAM expression is also developmentally regulated, with mRNA levels increasing in mouse in the two weeks immediately after birth, until they reach adult levels [61]. At the subcellular level, PAM has been found to reside in the nucleus, but it is also present in the cytosol and in the neurites of nerve cells [60,62,63].

5. Functions

At the functional level, RCC1 is undoubtedly the best characterized member of the family. RCC1 is a guanine nucleotide exchange factor (GEF) for Ran (*Ra*s-related *n*uclear) [for review on GEFs, see [64]]. The fact that Ran-GEF, i.e. RCC1, is in the nucleus whereas Ran-GAP (Ran GTPase activating protein) is in the cytoplasm gives rise to a gradient of the active form of Ran that acts as the driving force behind vectorial nucleocytoplasmic transport [65]. By contrast, during cell division the same gradient is established around mitotic chromosomes, to which RCC1 binds by means of its interaction with DNA and histones [23,27,31,66], an interaction that allosterically regulates RCC1's GEF activity [31]. This mitotic gradient, in turn, has a critical role first in mitotic spindle formation and then in nuclear envelope reassembly [67,68]. Unlike other RLD-containing proteins, RCC1 seems to be present in all eukaryotic organisms, including the baker's yeast *Saccharomyces cerevisiae*, where it is known as Prp20p or Srm1 [69–73], and the fission yeast *Schizosaccharomyces pombe*, where its name is Pim1 [74,75]. These RCC1 orthologs seem to be the only RLD-containing proteins in these organisms, and their functions appear to be very similar to their mammalian counterparts, as shown by the fact that these proteins also act as GEFs for the yeast orthologs of Ran [69–75]. Also consistent with a broad evolutionary conservation of RCC1's functions, both Prp20p and Bj1 (fruit fly's RCC1) can rescue the phenotype of RCC1 mutation in hamster cells [70]. This high degree of conservation suggests that functions so far only ascribed to some of the RCC1 orthologs, such as the role of Prp20p in nuclear pore complex (NPC)-mediated transcriptional control [76], may also be conserved in other organisms, although this remains to be proven.

Not so much is known about the other RLD-containing proteins. TD-60 is a member of the chromosome passenger complex (CPC) in inner centromeres and plays an essential role in prometaphase to metaphase progression [28], where it is required for proper localization of other CPC proteins (INCENP and Aurora B), for Aurora B kinase activation together with microtubules and for proper alignment of chromosomes on the metaphase plate [28,77]. TD-60 also seems to play a role in cytokinesis, but the mechanisms are not yet clear [30]. In addition, TD-60 has been shown to interact preferentially with the nucleotide-free form of Rac1, suggesting that TD-60 might be a Rac1-GEF, although this has not been demonstrated [28]. DelGEF regulates proteoglycan secretion by binding to DelGIP1 (DelGEF Interacting Protein 1) and to the exocyst component Sec5 [32,33], whereas WBSCR16 (also known as WBS16) is yet to be studied.

The HERC proteins have a HECT (*H*omologous to *E*6-AP *C*OOH-*t*erminus) domain and at least one RLD (for review see [5]). The HECT domain of these proteins is believed to be involved in the transfer of ubiquitin or ubiquitin-like proteins to target substrates [78]. In this respect, HERC5, which was initially described as Ceb1 [36], has been found to catalyze the transfer of ISG15 (interferon-stimulated gene-15), a protein related to ubiquitin, to a number of cellular proteins in response to interferon-α/β [38–40]. Moreover, HERC5 activity as an ISG15 ligase has been shown to be abolished both by mutation of the active site cysteine (Cys-994) in the HECT domain and by deletion of the RLD [38,39]. Apart from that, HERC5 mRNA has been shown to be induced by a number of inflammatory mediators, including LPS, TNF-α, IL-1β and IFN-α/β [37–39], whereas HERC5 protein levels are downregulated in response to LPS [37]. The HERC5 protein also interacts with both cyclin E and p21 and its levels rise in response to compromised p53 or retinoblastoma (Rb) activity, which suggests a role in the cell cycle [36]. Whether or not the roles of HERC5 in inflammation and the cell cycle are connected with each other has not been established yet. Interestingly, the HERC5 gene has only been found in primates, so it is unclear whether its functions are primate-specific or, alternatively, they are performed in other species by its close homolog HERC6 [7]. Consistent with the latter hypothesis, HERC6 is present in rodents and is also regulated by interferon-β [7,38].

HERC1, formerly known as p532, has been shown to bind to the tumor suppressor TSC2 and it has been suggested that HERC1 could be involved in TSC2 ubiquitination, but this has not been proven [79]. On the other hand, in vitro studies have shown that the HECT domains of both HERC1 and HERC3 form covalent thioester bonds with ubiquitin, suggesting that they have E3 ubiquitin ligase activity, but no target has been found in either case [41,80]. Furthermore, the RLD1 of HERC1 has been shown to associate with phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$), an interaction that is required for the ability of this domain to act as a guanine nucleotide release factor (GRF) for small GTP-binding proteins of the ARF and Rab families [4,42]. In addition, binding of the RLD1 to $PI(4,5)P_2$ has been suggested to be responsible for the recruitment of HERC1 onto actin-rich membrane protrusions [43]. HERC1 RLD2, on the other hand, interacts directly with clathrin heavy chain (CHC), which suggests a role for HERC1 in vesicular transport [44]. Although no biochemical studies exist concerning the function of HERC2, the phenotype of HERC2 mutant mice suggests a possible role for this protein in either vesicular trafficking or the cell cycle of specific cell types, but this awaits demonstration [34]. Regarding HERC3, in addition to having an active HECT domain [41,80], this protein has been shown to undergo ubiquitin-mediated proteasomal degradation and to non-covalently interact with ubiquitin [41]. However, even though its intracellular localization suggests a role in vesicular trafficking, nothing is yet known about the precise function of this protein [41]. Since HERC3 is most closely related to HERC4, it is possible that both proteins share similar functions. HERC4 has recently been identified as an important player in spermatogenesis [45]. More precisely, the absence of HERC4, which is normally expressed in all sperm cell precursors but not in mature spermatozoa, causes a significant reduction in sperm cell motility, which is due to incomplete clearance of proteins and organelles from sperm cell precursors, resulting in abnormal flagella in the mature sperm [45]. Although it seems very likely that the role of HERC4 in spermatogenesis is based on its ubiquitin ligase activity, this has not been demonstrated [45].

In the kinase subgroup, Nek9 is a p34/Cdc2 substrate with critical roles in cell cycle progression, both during interphase [47] and during mitosis [48,81]. In addition, Nek9 binds to Ran through both its RLD and kinase domains, but Ran-GEF activity like that of RCC1 has not been demonstrated [48] and it is thought to be unlikely given the relatively low similarity between the RLDs of both proteins [48]. Instead, the RLD of Nek9 is thought to play an autoinhibitory role by direct binding to the kinase domain [48,82,83]. Finally, the C-terminus of Nek9 has been shown to interact with Nek6 and Nek7, two members of the Nek family of kinases that, unlike Nek8 and Nek9, do not have RLDs [81]. In contrast to the cell cycle function of Nek9, Nek8 appears to regulate primary cilia length in kidney epithelial cells [84], which in turn may be a consequence of Nek8's ability to affect the phosphorylation status of other proteins, such as polycystin-2, a ciliary protein [85], or Bicd2, a protein involved in microtubule-dependent transport [50,86]. In the RCBTB subgroup, IBtk acts as an inhibitor of Bruton's tyrosine kinase (Btk) in lymphocytes [52], but nothing else is known about these proteins.

Concerning the last subgroup, Alsin was initially reported to act as a GEF for Rab5, via its Vps9 domain, and for Rac1, via its DH–PH domain [58]. Nevertheless, while the role of Alsin as Rab5-GEF seems to have stood the test of time, the same cannot be said for its purported Rac1-GEF activity, which is a source of controversy [59]. Indeed, a recent report suggests that Alsin acts as a downstream effector of active Rac1, rather than as a Rac1 upstream activator [59]. According to this study, Rac1 activation recruits Alsin to membrane ruffles and macropinosomes, which in turn leads to Alsin-mediated Rab5 activation and subsequent Rab5-dependent fusion of macropinosomes with other endosomes. Further evidence favouring a role for Alsin in the control of endocytic events comes from a number of additional studies [87–89]. In particular, Alsin's RLD has been shown to bind to GRIP1 (Glutamate receptor-interacting protein-1) and thereby control surface expression of AMPA-type glutamate receptors in neurons [89]. Moreover, neurons from $Alsin^{-/-}$ mice show defects in ligand-induced TrkB and IGF-1R endocytosis, which may be due to faulty Rab5-dependent endosome fusion [88]. Finally, Alsin's RLD has been shown to interact with the C-terminal half of Alsin (encompassing its MORN and Vps9 domains) and this has been proposed to play a role in the regulation of Alsin's translocation to the plasma membrane, but this remains unproven [59].

Concerning RPGR, mounting evidence suggests that it has an important role in regulating microtubule-dependent transport along cilia (see [9] for review). First of all, RPGR is located in cilia in different tissues (see Tissue and subcellular localization) and interacts, directly or indirectly, with a number of axonemal and basal body proteins, as well as with microtubule motor proteins [9,53,55,90–93]. Moreover, mutations in RPGR lead to malfunction of cells/tissues that depend on ciliary function for their proper operation, such as retinal photoreceptors (which require transport along their connecting cilia in order to properly assemble the light-capturing discs on their outer segments), airway epithelia or sensory cells in the cochlea (see Involvement in diseases). Apart from this, RPGR has been shown to associate, via its RLD, with the delta subunit of rod cGMP-phosphodiesterase, but the functional significance of this interaction is not entirely clear [94].

As its name reveals, PAM (protein associated with Myc) was identified by virtue of its binding to the transcriptional activation domain of Myc [60]. More recently, alternative splicing of PAM has been shown to regulate its binding affinity for this transcription factor [10]. The PAM–Myc interaction takes place in the nucleus, where PAM is enriched [60], and, although nothing is known about the functional significance of this interaction, a possible role is suggested by the fact that PAM is downregulated in quiescent and senescent fibroblasts relative to replicative ones [95]. Apart from the nucleus, PAM is also found in the cytoplasm, where it has been shown to act as a potent inhibitor of certain adenylyl cyclase (AC) isoforms [96]. In particular, when HeLa cells are treated with sphingosine-1-phosphate, PAM translocates from the endoplasmic reticulum to the plasma membrane and this results in a long-lasting inhibition of AC and of agonist-stimulated cAMP accumulation [97]. Remarkably, the RLD of PAM is sufficient for AC inhibition [96], and different residues within the RLD have been implicated in AC binding and inhibition, respectively [98]. In addition to its roles in the nucleus and in controlling cAMP-

mediated signaling, PAM has other tissue-specific functions. First, a role for the murine ortholog of PAM in the male germline is suggested by the fact that PAM mRNA contains a 26-nucleotide sequence that binds to DAZL, a germ cell-specific protein with an essential role in sperm production [99]. Although this suggests that PAM protein translation might be regulated during spermatogenesis [100], this has not been demonstrated. By contrast, plenty of evidence shows that PAM and its orthologs have a conserved role in neuronal development and function (for a review, see [101]). In mammals, apart from its higher expression in specific parts of the brain [61] and its role in spinal nociceptive processing, which may be dependent on AC inhibition [102], PAM (known as Phr1 in mouse) has recently been found to play an important role in motor axon navigation in the developing embryo [62]. This function has been connected with Phr1's ability to downregulate DLK, a MAPKKK, and thus block p38 MAPK signaling within axon shafts, which in turn controls the microtubule cytoskeleton [62]. These findings are consistent with previous results suggesting that Phr1 deletion underlies defects in respiratory muscle innervation leading to death of newborn mice [103] (see Involvement in diseases). Furthermore, PAM has been found to colocalize and interact with the TSC1/TSC2 tumor suppressor complex in the neurites and growth cones of brain cortical neurons [63]. This interaction is likely to be evolutionarily conserved, since the *Drosophila* and zebrafish orthologs of PAM have also been shown to functionally interact with this complex [63,104]. In zebrafish, PAM ortholog Esrom is required for the bundling and targeting of retinal axons [104] as well as for tetrahydrobiopterin and yellow pigment production [105]. In *Drosophila*, mutations in the PAM ortholog Highwire lead to increased synaptic growth but also to impaired synaptic function [106,107]. This phenotype is similar to the one observed in *C. elegans* when RPM-1, the worm ortholog of PAM, is mutated [108,109]. Indeed, recent studies have shown that both Highwire and RPM-1 control synaptogenesis in part by inducing the ubiquitination and subsequent degradation of target proteins in the synapse [110–116]. In the nematode, RPM-1 has been shown to be a constituent of a synaptic SCF-like ubiquitin ligase complex including Skp1, Cullin and the F-box protein FSN-1, with which RPM-1 interacts [114]. This complex regulates synaptic cytoarchitecture by mediating the degradation of DLK-1, a MAPKKK, and thereby suppressing signaling through a MAPK cascade involving DLK-1, MKK-4 (MAPKK) and PMK-3 (a p38 MAPK) [115]. Much of this pathway seems to be conserved in the fly (and probably in mouse also, see above), where Highwire also collaborates with DFsn (FSN-1 ortholog) [113] to induce degradation of Wallenda/DLK (DLK-1 ortholog) [112]. However, instead of suppressing a p38 MAPK cascade like RPM-1 does, Highwire suppresses activation of JNK and of the Fos transcription factor [112]. Nevertheless, suppression of MAPK signaling only explains part of the synaptic defects of RPM-1/Highwire loss-of-function mutants [107,112,116,117]. Thus, Highwire also acts by suppressing BMP signaling, which it probably achieves by inducing the ubiquitin-mediated degradation of the co-Smad protein Medea [117]. On the other hand, other downstream targets of RPM-1 include the GLO-4/GLO-1 pathway [116] and possibly the receptor tyrosine kinase ALK [114]. Interestingly, the N-terminal half of RPM-1, which includes its RLD but not its RING finger necessary for ubiquitin-dependent functions, has been shown to interact with GLO-4, itself an RLD-containing protein which in turn regulates the function of a Rab GTP-binding protein named GLO-1 [116]. This RPM-1/GLO-4/GLO-1 pathway, which acts in parallel with the RPM-1/DLK-1/MKK-4/PMK-3 one, regulates synaptic physiology by affecting late endosome function [116]. Although GLO-4, a protein most closely related to RPGR, DelGEF and *Drosophila* Claret, is a candidate GLO-1-GEF, this has not been demonstrated [116].

To finish this section, the available information on RCC1 superfamily proteins lacking clear mammalian orthologs will be briefly reviewed. As has been said, GLO-4 is most closely related to *Drosophila* Claret. Both GLO-4 and Claret have been shown to act upstream of Rab proteins (GLO-1 and Lightoid, respectively) and both are needed for the formation of lysosome-related organelles (gut granules in the case of GLO-4, eye pigment granules in the case of Claret) [118,119]. In addition, the RLD of Claret has been shown to bind preferentially to the nucleotide-free form of Lightoid, suggesting that Claret may act as a Lightoid-GEF. Whether Claret/Lightoid share the function of GLO-4/GLO-1 at the synapse remains to be determined. PARF-1 is an *Arabidopsis thaliana* protein containing PH, RLD and FYVE domains that has been shown to bind monophosphorylated phosphoinositides [120]. UVR8 (UV-resistance locus-8) is another *Arabidopsis* protein that, like RCC1, consists of a canonical RLD flanked by short N- and C-terminal tails (curiously, though, the closest mammalian homolog of UVR8 RLD is the RLD of HERC4, which is 38% identical). UVR8 accumulates in the nucleus in response to UV-B light and orchestrates the plant UV response by binding to chromatin via histones and inducing the expression of downstream genes such as HY5 and those involved in flavonoid biosynthesis [121–123]. Nd6p (non-discharge protein-6) is found in the protozoon *Paramecium tetraurelia* and presents two RLDs on its N-terminus [124]. Nd6p is required for dense core granule exocytosis and the RLDs seem to be essential for this function [124]. In bacteria, the Pfam database finds 65 proteins with canonical RCC1 repeats. Although none have been described yet, it will be interesting to see what proteins like Q74DR4 (Swissprot accession number), with more than 2000 amino acid residues and six RCC1 repeats in its N-terminus, are doing in bacteria such as *Geobacter sulfurreducens* [18,19]. Even a few viruses seem to contain RLD proteins, as is the case for neseORF72 and neseORF73, found in *Neodiprion sertifer* nucleopolyhedrovirus [18,19].

6. Involvement in diseases

Mutations in RCC1 superfamily proteins have been linked to several diseases. Among these, one of the best studied cases is that of RPGR, mutations of which are responsible for 70–80% of cases of X-linked retinitis pigmentosa (XLRP), one of the most severe forms of human retinal degeneration [9]. More than 200 disease-causing mutations have been identified in RPGR, many of them affecting its RLD. The vast majority of RPGR mutations (~95%) lead to XLRP, whereas the remaining 5% include other kinds of retinal degeneration and also, in four cases, ciliary dyskinesia, a disease whose symptoms include upper respiratory tract and middle ear infections as well as hearing loss [9]. All these phenotypes, together with the functional data reviewed above, are consistent with a role for RPGR in microtubule-dependent transport along cilia [9].

Alsin was discovered as the gene encoded by the ALS2 locus, which had been linked to an autosomal recessive form of juvenile-onset amyotrophic lateral sclerosis (jALS) [125,126]. To this day, ten disease-causing mutations have been identified in the Alsin gene, all of which lead to the production of truncated proteins (see [127,128] for reviews). Interestingly, in those cases where truncations are very early in the protein, affecting its RLD domain, these mutations lead to jALS, a condition where both upper and lower spinal cord motoneurons degenerate. By contrast, less severe truncations in Alsin lead to either juvenile primary lateral sclerosis (jPLS) or to infantile-onset ascending hereditary spastic paraplegia (IAHSP), in which only upper motoneurons are affected [127]. Surprisingly, Alsin$^{-/-}$ mice do not show obvious motor deficits [129,130], but their neurons appear to be more susceptible to oxidative stress [129]. In this regard, a critical connection between Alsin, oxidative stress and ALS may lie in the fact that Alsin binds to Cu/Zn superoxide dismutase (SOD1), the protein encoded by the ALS1 locus involved in autosomal dominant ALS [131,132]. Furthermore, Alsin has been shown to antagonize the neurotoxic effects of dominant SOD1 mutants, an activity for which the DH–PH domain of Alsin is essential [131,132]. Finally, Alsin$^{-/-}$ mice have recently been shown to have lymphopenia and other hematopoietic abnormalities resembling those also observed in ALS

patients [133,134]. Although this may point to a role for immunodeficiency in the ethiopathogenesis of ALS, this is not yet clear.

Another RLD-containing protein involved in disease is Nek8. In addition to being overexpressed in human breast tumors [135], a mutation in the RLD of Nek8 (G448V) was found to underlie a form of murine autosomal recessive polycystic kidney disease (PKD) known as juvenile cystic kidney, or jck [46,136]. Nek8 localization to cilia is lost in the renal epithelia of these animals, which results in structurally abnormal cilia and leads to cyst formation [84,137]. Although little is known about the detailed mechanisms controlling cystogenesis, this process appears to be regulated by a pathway involving both Nek8 and Pkd1, another protein involved in PKD [138]. On the other hand, mutations in human Nek8 have very recently been identified as the cause of nephronophthisis type 9, a juvenile renal cystic disease [139], and it has been suggested that Nek8 might also be linked to human PKD [140].

Also involved in mouse pathology is HERC2, mutations of which lead to the rjs syndrome (runty, jerky, sterile), also known as jdf2 (juvenile development and fertility-2) [34,141,142]. Although the pathogenic mechanisms of this syndrome are not known, it has been suggested that at least some of its symptoms could be due to pituitary defects [34]. In humans, the HERC2 genomic locus, including multiple partially duplicated paralogs of HERC2 [143], corresponds to the chromosomal breakpoint region in deletions causing Prader–Willi and Angelman syndromes [144,145]. However, lack of HERC2 protein does not seem to play a role in any of these syndromes [142]. Likewise, in a very recent set of papers, a single nucleotide polymorphism (SNP) in intron 86 of the HERC2 gene has been shown to determine human blue/brown eye color, but this intronic sequence has its effects by controlling the expression of the neighboring OCA2 gene [146–148].

Concerning other HERC proteins, HERC1 was found during the course of a screen aimed at identifying mammary oncogenes and was shown to be overexpressed in tumor cell lines relative to normal ones [4]. More recently, HERC1 has been shown to bind to the tumor suppressor TSC2 [79]. However, in spite of these data, it remains unclear whether HERC1 plays any role in tumorigenesis.

On the other hand, HERC5 seems to be an important player in the innate immune response [37–39]. Initially, HERC5 expression in endothelial cells was found to be tightly modulated by proinflammatory cytokines such as TNF-α and IL-1β and by the bacterial lipopolysaccharide (LPS), suggesting a role for HERC5 in inflammation and bacterial infections [37]. More recently, HERC5 levels have also been shown to be upregulated by type I interferons, which are themselves expressed in response to bacterial or viral infections [38,39]. Furthermore, it has been shown that, in response to interferons α or β, HERC5 catalyzes conjugation of ISG15, a ubiquitin-like protein, to a broad spectrum of target proteins [38–40]. Although the exact function of ISG15 conjugation, also known as ISGylation, to target proteins is not yet known, it seems clear that this is an important innate defensive mechanism, as supported by the fact that a viral protein, NS1 from influenza B virus, has developed the ability to specifically block ISGylation [149,150].

Apart from the possible presence of anti-PAM autoantibodies in some schizophrenic patients [151], no evidence exists that PAM is involved in any human disease. Nevertheless, Phr1, the murine ortholog of PAM, is the best candidate for a respiratory distress syndrome that kills mice at birth [100]. In these mice, the phrenic nerve fails to completely innervate the diaphragm, whereas the intercostal muscles, though innervated, show dysmorphic nerve terminals [100]. This phenotype is consistent with both the evolutionarily conserved role of Phr1 orthologs in synaptic development [110,112–115] and its effects on motor axon navigation in the embryo [62]. Interestingly, Hirschsprung's disease in humans is often accompanied by congenital central hypoventilation syndrome and it has been proposed that this might be due to genomic deletions encompassing not only the Ednrb gene (responsible for Hirschprung's disease) but also the neighboring PAM gene [100].

WBSCR16, whose name comes from the fact that the gene is located in the Williams–Beuren syndrome (WBS) critical region, is one of many genes at 7q11.23 one copy of which is deleted in this neurodevelopmental disorder. WBS is thought to arise as a result of haploinsufficiency of multiple genes in this region and the precise contribution, if any, of WBSCR16 to the WBS phenotype is not known [152].

DelGEF was thus named because of its genomic location in a deafness locus (DFNB18) and its assumed role as a GEF. While there is no evidence of DelGEF having GEF activity, it has been proven that the gene responsible for non-syndromic recessive deafness (DFNB18) is not DelGEF, but USH1C [153]. Therefore, there is no evidence connecting DelGEF with any human disease.

Finally, the RCBTB1 and 2 genes, formerly CLLD7 and CHC1L respectively, are located very close to each other in chromosomal band 13q14. RCBTB1 is a candidate tumor suppressor gene for B-cell chronic lymphocytic leukemia (B-CLL) [154–156], whereas RCBTB2 might be a tumor suppressor in sporadic prostate cancer [157].

7. Conclusions

The initial identification of RCC1 as the guanine nucleotide exchange factor (GEF) for Ran led many to assume that RCC1-like domains (RLDs) in other proteins would also act as GEFs for small GTP-binding proteins. Although this is clearly the case for other domains with GEF activity [64], it does not seem to hold true for RLDs. Indeed, it is becoming clear that different RLDs, rather than sharing a conserved function, share a very versatile conserved structure that has been co-opted by a number of proteins to perform many different functions.

Most often, RLDs participate in protein–protein interactions. These interactions do sometimes involve small GTP-binding proteins, but only in the case of RCC1 has GEF activity been shown conclusively. Although some other RCC1 superfamily proteins are certainly good GEF candidates (e.g. Claret and GLO-4), it remains a mystery how many of them will eventually turn out to be bona fide GEFs. In other cases, binding of an RLD to a target protein may affect protein localization (e.g. RPGR targeting to the connecting cilium depends on its RLD binding to RPGRIP) or may affect the activity of the target protein (e.g. PAM's RLD inhibiting adenylyl cyclase activity). Apart from binding to other proteins, RLDs have also been shown to bind to lipids (the case of HERC1), which underscores the functional versatility of this domain.

It has been more than twenty years since RCC1 was originally identified. Over these two decades, all human RCC1 superfamily proteins, and many non-human ones, have been discovered and most of them are characterized to some extent. In practically all cases, however, the functions of these proteins are only beginning to be understood, which guarantees many fascinating discoveries for the near future.

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