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Review

Leucine-Rich Repeat Domains in the Class III Adenylyl Cyclase of Fungi: Some Features of the Sequences and Structures

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Abstract

Fungal adenylyl cyclase (AC) converts adenosine triphosphate (ATP) to the second messenger 3′,5′-cyclic AMP (cAMP). Class III ACs contain multiple domains, including the adenylyl cyclase domain and a leucine-rich repeat domain (LRR), through which multiple signals are integrated into the cAMP-Protein Kinase A (PKA) signaling response. This review focuses on the sequences and structural features of the LRR domains. To this end, we analyzed LRR domain sequences from ACs in the phyla *Ascomycota*, *Mucoromycota*, *Basidiomycota*, *Blastocladiomycota*, *Zoopagomycota*, and *Chytridiomycota*. Protein structures were also predicted using AlphaFold2. More than 99% of individual LRR domains consist of 23 repeats with an additional parallel β-strand at the C-terminus. The repeat unit length (RUL) is mainly 23 or 24 residues. The most distinctive feature is the presence of non-LRR island region (IR) interrupting the LRRs between the 16th and the 17th units. The IRs may be roughly classified into two groups. The first group IRs, which, for example, are identified in ACs from *Saccharomyces cerevisiae* and *Candida albicans* belonging to the phylum *Ascomycota*, are relatively short; the IR lengths are within 10 to 50 residues. The second group IRs, which are identified in many ACs from the phyla *Ascomycota*, *Mucoromycota*, *Basidiomycota*, and *Zoopagomycota* are long; the IRs range from 50 to 220 residues. Most of the long IRs are regarded as intrinsically disordered regions. The AC LRR domains adopt typical solenoid structures in which the IRs form bulges or loops in a non-globular conformation. Two possibilities are discussed for the role of IRs in AC LRR domains. This review proposes a new subclass of fungal class III ACs, which includes a forkhead-associated domain.

Keywords: fungal class IIIc adenylyl cyclase; leucine-rich repeat; island region (IR); intrinsically disordered region (IDR); solenoid structure; forkhead-associated domain (FHA)

1. Introduction

Adenylyl cyclase (AC) converts adenosine triphosphate (ATP) to the second messenger 3′,5′-cyclic AMP (cAMP) [1]. Fungal ACs belong to the class III family. The class III ACs have been classified into three subclasses of IIIa, IIIb, and IIIc [2]. The IIIc ACs are large multi-domain enzymes including leucine-rich repeat domain (LRR) [3,4]. The subclass IIIc ACs of *Saccharomyces cerevisiae* and *Candida albicans* possess six conserved domains, from the N- and C-terminus, including a Gα-binding domain (GαBD), a Ras-association domain (RA), an LRR, a protein phosphatase 2C (PP2C) domain, an adenylyl cyclase catalytic domain (ACD), and a Cap1 (cyclase-associated protein1) binding domain (CBD) (Fig. 1) [3,5]. The CBD domain consists of three conserved motifs of which motifs II and III are required for binding [6]. Through their domains, multiple signals are integrated into cAMP-PKA signaling response [3,4]. The cAMP signaling pathway can regulate fungal growth, development, and virulence [7,8,9]. Fungal ACs play an important role in biocontrol [10,11,12,13,14].

LRRs occur in tandem in many proteins. The LRR domains act as scaffolds for protein-protein/ligand interactions and are crucial for immune responses, cell signaling and plant growth/defense [15,16,17]. The repeating unit length (RUL) is separated into a highly conserved segment (HCS) and a variable segment (VS) [18]. For ease of explanation, we use the following abbreviations. The HCS part with eleven residues is abbreviated as HCS = 11 for ease of explanation [18]. The HCS part in most LRR types is represented by the consensus of LxxLxLxxNxL with HCS = 11 or LxxLxLxxCxxL with HCS = 12, in which “L” is Leu, Val, or Ile, “N” is Asn or Thr, “C” is Cys or Asn, and “x” is any amino acid. Nine types have been well recognized as RI-like, Cysteine-containing, Gala, SDS22-like, Plant specific, Leptospira-like, Bacterial, IRREKO, and Typical [19,20,21,22]. We called these types canonical LRR, as some non-canonical types were identified [23]. The canonical LRRs mostly form three-residue, short β-strands at positions 3–5 in the HCS part, while the VS part adopts various secondary structures such as α-helix, 3(10)-helix, polyproline II helix, and consecutive β-turns. The β-strand parallel stacking occurs and consequently produces super helix arrangement called solenoid structure. The overall shape resembles a horseshoe or an arc [24,25]. The solenoid struc-

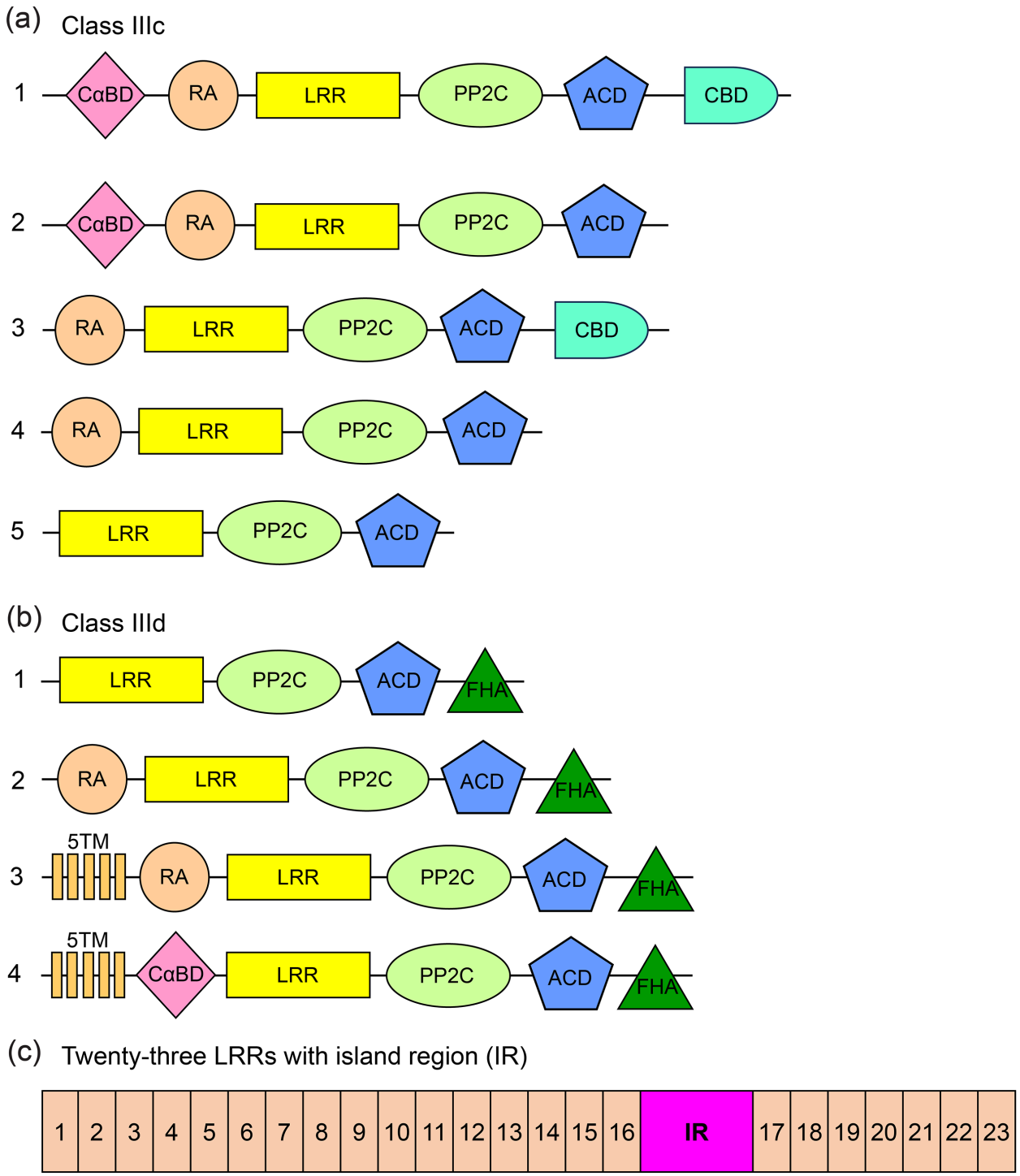

Fig. 1. Schematic of domain architecture of fungal class III adenylyl cyclase (AC) and the island region (IR) within LRR domains. (a) AC class IIIc, (b) AC class IIId, (c) IR. Domain labels: Gα-binding domain (GαBD), a Ras-association domain (RA), leucine-rich repeat domain (LRR), protein phosphatase 2C domain (PP2C), an adenylyl cyclase catalytic domain (ACD), and a Cap1 (cyclase-associated protein1) binding domain (CBD), forkhead-associated domain (FHA), Transmembrane domains (consisting of five helices) (TM).

tures consist of four parts which are a concave surface, ascending loops, a convex surface, and descending loops [22].

Island region/domain interrupting LRRs (referred to as an "island" or "loop out") (IR) are observed in many LRR proteins including LRR-containing receptor-like kinase (LRR-RLK) and LRR-containing receptor-like protein (LRR-RLP) from plants, three members of the Toll-like receptor (TLR) family (TLR7, TLR8, and TLR9) from

vertebrates, and LRRC32 (GARP) and LRRC33 from human [26,27,28,29,30]. Furthermore, we reported that fungal ACs have IRs within LRRs [29]. The structures of LRR proteins containing IR are available for the Arabidopsis BRI1 [31,32], Arabidopsis PSKR [33], Nicotiana RXEG1 [34], human TLR7, TLR8 and TLR9 [35,36,37], human LRRC32 (GARP) [38,39,40,41], and human LRRC33 [42]. BRI1 and PSKR belong to LRR-RLKs. BRI1 is the primary brassinosteroid (BR) receptor, while PSKR is the phytosulfokine (PSK) receptor; PSK is a small peptide hormone with five amino acids. The AtBRI1 LRR domain forms a right-handed superhelix composed of twenty-five LRRs interrupted by a 70-residue IR between *LRR21* and *LRR22*. The IR, together with *LRR22*, binds to BR [31,43,44]. AtPSKR contains twenty-two LRRs interrupted by a 36-residue IR between *LRR18* and *LRR19* [32]. PSK binding causes the receptor's flexible IR regions to fold, forming a stable cavity that traps the hormone. This complex is perfectly shaped for the co-receptor to bind. In the unbound receptor, the IRs are too flexible to be seen in the structure. RXEG1 to be an LRR-RLP recognizes a pathogen glycoside hydrolase, XEG1. Nicotiana RXEG1 contains thirty-two LRRs with a 49-residue IR between *LRR28* and *LRR29*. The crystal structure of XEG1 – RXEG1 (LRR) revealed a specific interaction between XEG1 and two loop out regions of RXEG1 (LRR)—an N loop out, and the 49-residue IR - [34,45]. TLR7 and TLR8 bind to ssRNA, while TLR9 binds to CpG DNA [46]. All of TLR7, TLR8 and TLR9 contain twenty-six LRRs. The IR in these TLRs is called Z-loop that consists of approximately 30-40 amino acids between *LRR14* and *LRR15* [46]. The protease cleavage of the Z-loop was required for proper functioning of these proteins [47,48]. Both LRRC32 (GARP) and LRRC33 are a TGF-β receptor [49,50]. GARP contains 22 LRRs. The eleventh unit is 57 residues long, while the remaining range from 21 to 33 residues. Similarly, the corresponding LRR unit in LRRC33 is 56 residues long. Thus, the IRs with ~20 residues are present between *LRR11* and *LRR12* [29]. The structures revealed that the IRs were not present in the electron density map due to disorder [38,39,40,41,42]. The IR's role is still unknown.

In this review we focus on some features of the sequence and structure of the AC LRR domain of fungi. The kingdom Fungi comprises nineteen phyla [51]. Here we performed the sequence analysis of LRR domains in ACs from the phyla *Ascomycota*, *Basidiomycota*, *Blastocladiomycota*, *Zoopagomycota*, *Mucoromycota*, and *Chytridiomycota*. The protein data sequences were collected using the UniProt database [52]. We also predicted the structures by AlphaFold2 [53,54,55]. Over 99% of individual LRR domains consist of twenty-three repeats with an additional parallel β-strand at the C-terminus. The repeating unit length (RUL) is mainly 23 or 24 residues. The most distinctive feature is the presence of non-LRR IR between the 16th and the 17th units. The IRs may be roughly classified in two groups. The first group IRs, which, for example, were identified in ACs from the phylum *Ascomycota*, *S. cerevisiae* and *C. albicans*, are relatively short; the IR lengths are within 10 to 50 residues. The second group IRs, which are identified in many ACs from the phyla *Ascomycota*, *Mucoromycota*, *Basidiomycota*, and *Zoopagomycota*, are long; the IR lengths are within 50 to 220 residues. Most of the long IRs are regarded as intrinsically disordered region. The AC LRR domains adopt typical solenoid structures as seen in many other LRR proteins. The IRs form bulges or loops in non-globular state. Two possibilities are discussed for the role for IRs in AC LRR domains. We propose a new subclass (IIId) of the class III ACs which includes a forkhead-associated domain (FHA) [56]. We hope that this review is useful for next research project.

2. Four Subclasses of the Class III AC of Fungi and Their Domain Architecture

The class III ACs of fungi have been classified into three subclasses of IIIa, IIIb, and IIIc [2]. The IIIa ACs contain five transmembrane regions, two cyclase transducer elements (CTEs), two ACDs, and a conserved mAC linker (C1b). The subclass IIIb ACs contain only an ACD. The subclass IIIc are grouped into five types depending on whether GαBD and CBD exist or not (Fig. 1a).

The fourth type of the subclass IIIc is the most common among the five types. The first type of the subclass IIIc possesses six conserved domains of GαBD, RA, LRR, PP2C, ACD, and CBD (Fig. 1) [4]. The well-known examples are *S. cerevisiae* and *C. albicans* ACs. These biochemical studies are well underway. GαBD binds to the α subunit (GPa2) of a G-protein [57]. The RA domains bind to Ras1 in *C. albicans* and to Ras2 in *S. cerevisiae* [58,59]. Ras1 in its GTP-bound form directly interacts with *C. albicans* AC and stimulates cAMP production [59,60,61]. The function of PP2C is yet not elucidated [59]. In *C. albicans* the AC domain which converts ATP to cAMP is also involved in CO_2 and farnesol sensing [6,62]. Examples of the second type are observed in ACs from the phylum *Ascomycota*, *Monilinia fructicola* (UniProtKB: A0A5M9J5C4) and the phylum *Basidiomycota*, *Cryptococcus depauperatus* (A0AAJ8JWY1). Examples of the third and fifth types are in ACs from the phylum *Ascomycota*, *Hanseniaspora uvarum* (A0A1E5RYF2) and from the phylum *Basidiomycota*, *Cryptococcus deneoformans* (Q5KDK1), respectively. Furthermore, the phylum *Ascomycota*, *Verticillium longisporum* encode exceptional ACs (A0A0G4LDT7 and A0A0G4KWA9) that likely belong to the class IIIc. The ACs that are large proteins contain two ACDs, although the sequences are incomplete.

The database search identified the class III ACs of fungi including a forkhead-associated domain (FHA) [56,63]. The FHA domains are phosphopeptide-binding modules that function in cell cycle regulation, DNA repair, and signaling [63]. The IIId ACs are observed only in

the subphylum *Wallemiomycotina* of the phylum *Basidiomycota* (R9AHL3, A0A4T0FWS0, A0A4T0I8R0, A0A4T0M8G6, A0A4T0J5D1, A0AB38N1H4, A0A4T0MDV5, and A0A4T0R5S9), while the class III AC from *Wallemia mellicola* (strain ATCC MYA-4683/CBS 633.66) (I4YHB8) does not contain FHA. We propose a new subclass AC (IIId) which is grouped into four types with or without transmembrane region or RA, GαBD (Fig. 1b).

3. Sequence and Structure of Fungal AC LRR Domain

Young et al. [64], first reported that *Schizosaccharomyces pombe* AC (Cyr1p) contains tandemly repeated motif rich in leucine. Xu et al. [65], noted that the repeated motif in *C. albicans* AC forms the evolutionarily conserved LRR domain consisting of fourteen repeats. We found non-LRR IR interrupting LRRs in fungal ACs [29]. Very recently, Crump et al. [66], performed the characterization of the entire region of the LRRs in *C. albicans* AC by optimal recombinant expression workflows. Circular dichroism (CD) spectroscopy indicated that the LRR domain is well folded and consists of 28.8% α-helix, and 16.4% β-strands. AlphaFold2 reveals that the secondary structure in visual agrees with the experimental observation from CD spectroscopy [66]. Here we performed the comparative sequence analysis of LRR domains in fungal ACs in more detail (Fig. 1).

3.1 Overall Structures of the AC LRR Domains

We predicted the structures of ACs by AlphaFold2 in fifteen representative examples [53,54,55]. The predicted structures show high pLDDTs (>90 and/or >70) in the AC LRR domains except for the IRs, while the IRs show low pLDDTs (<60). The AlphaFold2 structures reveal that the parallel stacking of a three-residue β-strand on the concave side produces a solenoid structure, as observed in many LRR proteins (Fig. 2, **Supplementary Figs. 1,2**) [24]. The overall shape is a distorted arc with a four-fifths turn. The IRs form a bulge or a large descending loop, as described later.

The occurrence of Trp cluster is generally rare in protein sequence. Individual LRR domains consist of 23 repeats, as described later. The VS parts of LRR21 are frequently rich in Trp. In some cases, the VS part in one AC (Q9P411) contains the sequence of WxYxWxWxWx (**Supplementary Fig. 1**). The AlphaFold2 structure predicts that Trp residues located internally form hydrophobic core, while Trp residues in the outside likely stabilize two loops in LRR21 and LRR22 through the Trp -Trp interactions (Fig. 2b).

3.2 The Repeat Number, Repeat Length and Types of LRR

The sequence alignment was based on both the HCS consensus and the AlphaFold2 structures (**Supplementary Table 1**). The repeat number of the LRRs (*N*) is 23 in over 99% of individual LRR domains, as far as we investigated; one additional parallel β-strand occurs at the C-terminus of the LRR domains. Fewer than $N = 23$ such as $N = 16$, 20, 21, and 22 are also observed in some ACs (R8BF45, C0NCV3, A0A1Y2I4J1, and A0A2T9Z515), which is likely due to the deletion. The third and fourth types of the subclass IIId ACs (A0A4T0I8R0, A0A4T0M8G6, A0AB38N1H4, and A0A4T0R5S9) contain only six or seven LRRs. The LRR sequences are very similar to those from *LRR17* to *LRR23* seen in the LRR domains in many ACs.

The repeating unit length (RUL) is mainly 23 or 24 residues with HCS = 11. The HCS parts of *LRR2* and *LRR22* are twelve residues long as opposed to HCS = 11. Two units (*LRR21* and *LRR22*) before the last one (*LRR23*) are longer 29–37 residues and 34-209, respectively. The central repeat (*LRR10*) is the shortest with 10 or 21 residues in the AC LRR domains except for those from the phylum *Chytridiomycota*. The VS parts with RUL = 23 are represented by xxLPxxLxxLxx which belong to Leptospira-like type and mostly adopt a seven-residue 3(10)-helix on the convex side, while those with RUL = 24 mainly adopt an α-helix or 3(10)-helix (Fig. 2 and **Supplementary Fig. 1**). The short VS parts with RUL = 20 or 21 frequently have the sequence of hxh in which "h" is a hydrophobic residue. The VS parts strongly prefer short β-strands.

3.3 Island Region (IR) Interrupting LRRs

The non-LRR IRs are present between *LRR16* and *LRR17* (Fig. 1c and **Supplementary Table 1**). The boundary between the VS part and the IR is generally vague. So tentatively, we separate the long RUL of *LRR16* into three parts consisting of HCS (= 11), VS (= 23) and IR (= the remaining sequence) from the N- and C-terminus. The IRs may be roughly classified in two groups. The first group IRs are relatively short; the IR lengths are within 10 to 50 residues. The short IRs were identified in ACs from all six phyla analyzed here. However, the ACs with short IRs are overwhelmingly small.

Among the first group, for example, in ACs from the phylum *Ascomycota*, *S. cerevisiae* and the phylum *Blastocladiomycota*, *Allomyces macrogynus* (A0A0L0SRM9), the IR lengths are very short, around 10 (**Supplementary Table 2**). Consequently, the IRs form small bulges on the convex side (Fig. 2a and **Supplementary Fig. 2a**). Some IRs are around 30 residues long in ACs, for examples, from *C. albicans* and *S. pombe* and *S. japonicus*, the phylum *Basidiomycota*, *Dioszegia hungarica* (**Supplementary Table 2**). The IRs form various bulges adopting partially α-helix on the convex side (Fig. 2b and **Supplementary Fig. 2b,c,d**), while a 27-residue IR in AC from the phylum *Zoopagomycota*, *Conidiobolus coronatus* is irregular (**Supplementary Fig. 2e**). The secondary structure assignment in the AlphaFold2 structur of the *C. albicans* LRR

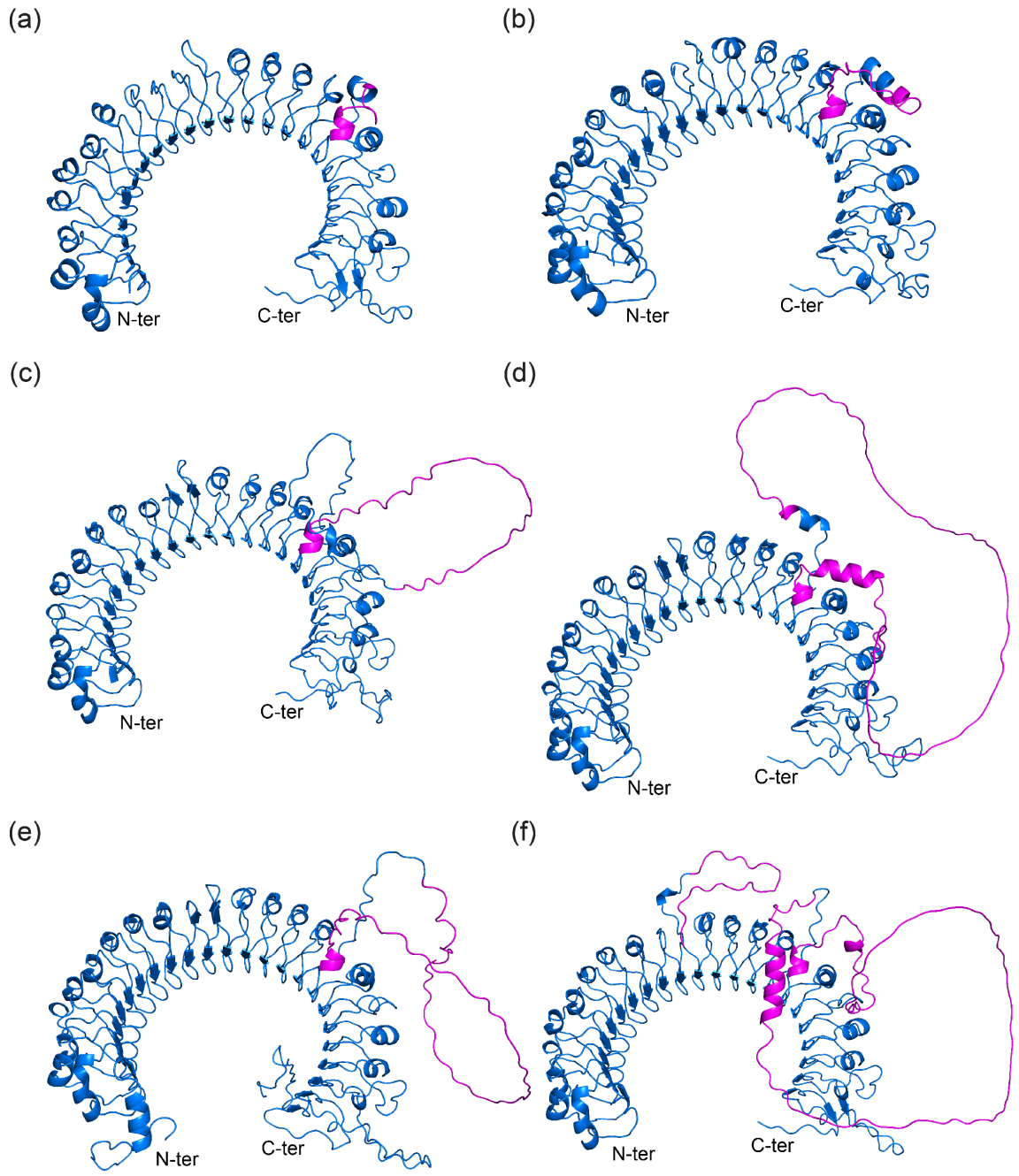

Fig. 2. The ribbon representation of the structures of the AC LRR domains in six representative species. (a) The phylum *Ascomycota*, *Saccharomyces cerevisiae* AC with 2026 residues (P08678). The IR in the AC LRR domain is between the 16th and 17th LRR units (abbreviation, *LRR16*-IR-*LRR17*). The IR length is 10 residues (abbreviation, IR = 10). The IR forms a small bulge with short α-helix. (b) The phylum *Ascomycota*, *Candida albicans* AC with 1690 residues (Q9P411), *LRR16*-IR-*LRR17* with IR = 22. The IR forms a bulge with two short α-helices. (c) The phylum *Basidiomycota*, *Pseudozyma sp. GHG001*AC with 2516 residues (V5EXV1), *LRR16*-IR-*LRR17* with IR = 66. The IR forms a large disordered loop. (d) The phylum *Ascomycota*, *Ramalina farinacea* AC with 2010 residues (A0AA43QNQ3), *LRR16*-IR-*LRR17* with IR = 107. The IR forms a large loop with α-helix. A large part of it is disordered. (e) The phylum *Ascomycota*, *Yarrowia lipolytica* AC with 2052 residues (Q6CE40), *LRR16*-IR-*LRR17* with IR = 75. The IR forms a large disordered loop. (f) The phylum *Mucoromycota*, *Lichtheimia corymbifera* AC (A0A068S166) with 1708 residues, *LRR11*-IR-*LRR12* with IR = 24 and *LRR16*-IR-*LRR17* with IR = 116. The first IR forms a disordered loop. The second IR forms a large loop with α-helix. A large part of it is disordered. Coiled ribbons show α-helices or 3(10)-helices. Arrows show β-strands/sheets. The parts of IRs are shown by magenta color. The other parts are shown by blue color.

domain showed that the content of α- and 3(10)-helices is 0.24, while β-strand content is 0.16 (**Supplementary Fig. 2b**), which appears to correspond to the CD experimental result [66].

The second group IRs are long; the IR lengths are within 50 to 220 residues. A representative example is seen in AC from the phylum *Ascomycota*, *Hymenoscyphus fraxineus* (A0A9N9L6X6). The IR is 113 residues long. The similarity search using the sequences of the VS part of *LRR16* and the IR as a query sequence detected IRs in nearly 1000 fungal ACs with significant similarity (**Supplementary Table 2**). The PONDR-FIT program [67] indicated that most of the IRs are intrinsically disordered regions (IDRs) [68]. Sequence alignment indicates that the VS parts of *LRR16* are very rich in proline and the first five-residue sequence is represented by xxFPK, while the IR sequence at the C-terminal side is well represented TF(A/G)(G/S)S. One of the homologs is *Ramalina farinacea* AC (A0AA43QNQ3) used for AlphaFold2 (Fig. 2d). The IR forms a large bulge or a large loop on the convex side. The FPKPPT sequence at the N-terminal side adopts a polyproline II helix and the TFAAS sequence at the C-terminal side adopts α-helix. The central part of the IR is entirely disordered. These structural features were seen in other IRs belonging to the second group (Fig. 2e,f, and **Supplementary Fig. 2g**). The long IRs do not form a globular domain.

In many cases, any IR sequence with significant similarity is limited to those in ACs from closely related species. Fig. 3 shows three examples. The most extreme case is seen in *Jaapia argillacea* AC from the phylum *Basidiomycota* with a 39-residue IR (A0A067PLN1). The similarity search detected only this IR.

Another IR is observed between *LRR11* and *LRR12* in the LRR domains in ACs from the phylum *Mucoromycota* (Fig. 2f and **Supplementary Fig. 2g**). Furthermore, multiple IRs are identified in ACs from the phylum *Chytridiomycota* (**Supplementary Fig. 2h,i**). Most of fungi belong to the subkingdom Dikarya (*Ascomycota* and *Basidiomycota*). Phylogenetic position of *Mucoromycota* and *Chytridiomycota* has been deeply investigated [69,70]. Strassert and Monaghan [69] showed a sister relationship of *Chytridiomycota* to all other non-*Opisthosporidia* fungi.

3.4 Mutations in the AC LRR Domain

In *S. cerevisiae* AC (ScCyr1), many mutations (deletions, insertions or point mutations) involving the LRR domain completely abolished the Ras-dependent activation of ScCyr1 [71,72]. A specific temperature-sensitive allele, *cdc35-1*, of *S. cerevisiae* AC (P08678) has a point mutation (L901H substitution) in the LRR domain [73,74]. The L901H substitution is located in *LRR5* that belongs to Leptospira-like type. The mutation substitutes one of the conserved leucine residues, thereby destabilizing the fold. The LRR motif is the putative site of Sgt1 interaction [74,75]. *Cryptococcus neoformans* AC (J9VV26) was found to have a single point mutation (R1227P) that confers a strong competitive advantage for growth inside macrophages, suggesting that mutations in this pathway are involved in host adaptation and can impact virulence [76]. The mutation is located in the HCS part in *LRR11*. Arg1227 is a non-conserved residue in the LRR HCS. This mutation likely preserves the solenoid structure.

4. The Role of the AC LRR Domain

4.1 Protein Interaction of the AC LRR Domain

In *C. albicans* AC, the LRR domain recognizes bacterial peptidoglycan (PGN) and muranyl dipeptides [65,66,77]. In *C. albicans* and *S. cerevisiae* the LRR domains also interact with their respective Hsp90/Sgt1 proteins *via* the co-chaperone, Sgt1 [74,75,78]. The LRR domain of *S. cerevisiae* AC (ScCyr1) interacts with the Ras protein [72,79,80]. Genetic and biochemical studies demonstrated that the LRR domain contains a binding site for the GTP-bound Ras [71,72,79,81,82,83].

S. cerevisiae AC has many other interactors including the HRAS protein [84]. Interactome analysis of *C. albicans* AC has been reported [85]. The LRR domain acts as a critical protein/ligand protein interaction interface and signal sensor. Thus, we cannot rule out the possibility that the LRR domain senses other molecules in other ACs. Soanes and Talbot [86] insisted that the kingdom Fungi does not encode genes of pattern recognition receptors such as TLRs in animals or LRR-RLKs including FLS2 in plants.

4.2 The Role of IRs in AC LRR Domains

The functional role of AC IRs is still unknown. Most of the long IRs in fungal ACs that are regarded as IDRs do not form globular domains. IDRs frequently participate in ligand interactions [87]. The IR in *Mucor plumbeus* AC (the phylum *Mucoromycota*) (A0A8H7QMI8) is 205 residues long, which is entirely an IDR. The IR contains polyglutamine (PolyQ) consisting of consecutive 57 glutamine residues (Q_{57}). The stretches of Q_{15} and Q_{17} are also observed in ACs (A0A8H4F1M3, and A0A0C9ME99). Furthermore, polyalanine (PolyA) is observed in some IRs (Fig. 3c). PolyQ and PolyA are often implicated in protein-protein interactions [88]. The IRs in plant LRR-RLKs, BRI1 and PSKR and plant LRR-RLP, RXEG1, interact directly with the respective ligands [31,34,43,44,45]. Functional roles for the IRs in plant LRR-RLPs such as Cf-9 and Cf-4 and LRR-RLKs such as RLP42 have been discussed and reviewed [89,90]. Based on the properties of IDRs and analogy to homologous proteins, the AC IRs might participate in interaction with unknown ligands/proteins.

There is another possibility. The IRs in BRI1 and PSKR, and RXEG1 are located on the concave surface of the solenoid structures. In contrast, the predicted structures show that the IRs in fungal ACs protrude from the convex surface (Fig. 2 and **Supplementary Fig. 2**). The con-

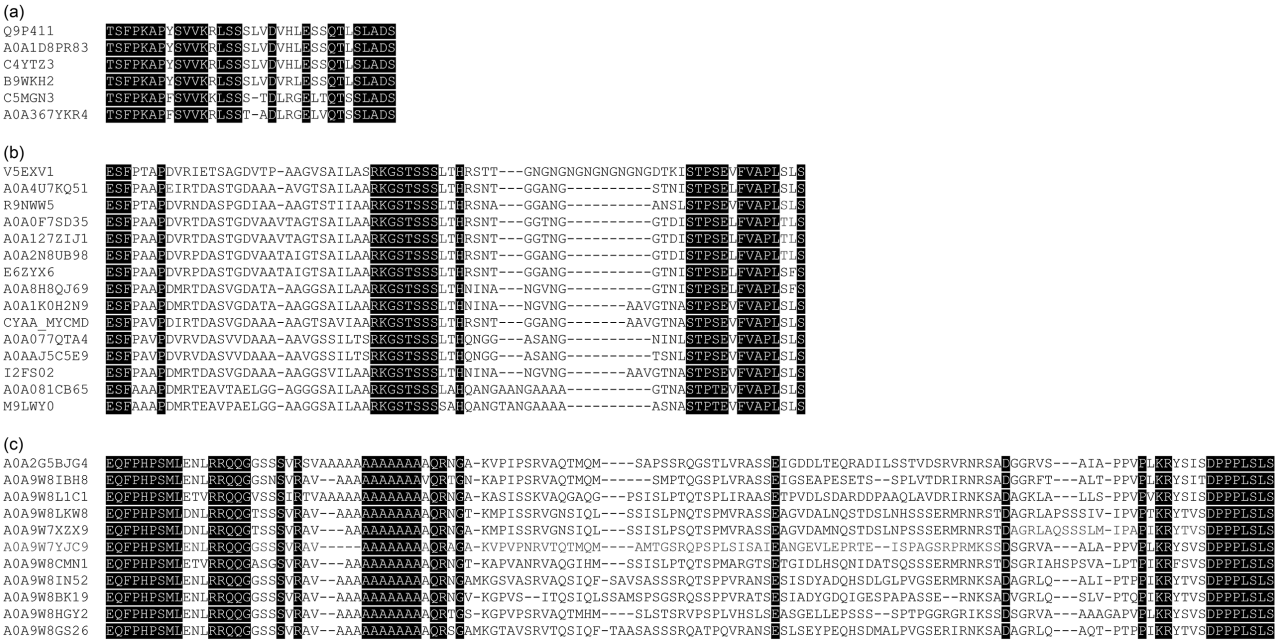

Fig. 3. Sequence alignment of the island regions (IRs) between *LRR16* and *LRR17* (including the VS of *LRR16*) within fungal AC LRR domains by the MAFFT program. Three examples are shown. (a) The IRs in *Candida albicans* AC (Q9P411) and the five homologs. (b) The IRs in *Pseudozyma sp. GHG001* AC (V5EXV1) and the fourteen homologs. (c) The IRs in *Coemansia reversa* AC (A0A2G5BJG4) and the ten homologs.

cave surface of the LRR domain is generally responsible for binding to ligands. In a few rare cases the convex face is involved in the ligand interaction [91,92,93]. The crystal structure of the complex with anti-PD-1 nanobody Nb52 is available as PDB entry 8XFS. X-ray crystallography shows that the IRs in LRRC32, LRRC33, and Nischarin [8ESF] are invisible in the electron density [38,39,40,41,42]. When the IRs (Z-loop) in TLR7, TLR8 and TLR9 are uncleaved, the receptor's ability to dimerize is limited [46,47,48]. Deletion or cleavage of this loop is the key step in relieving autoinhibition. This phenomenon might occur in the IRs in fungal AC LRRs, which would contribute to the function of ACs.

5. ACs in Opisthokonta and Plants

The Opisthokonta is a large supergroup of eukaryotes including fungi, metazoans, some flagellate (choanoflagellates), amoeboid (e.g., Nucleraria) and sporozoan (e.g., Ichthyosporea, Microsporidia) protists [94]. Three species attributed as the Opisthokonta—*Fonticula alba* (A0A058ZHN6), *Sphaeroforma arctica JP610* (A0A0L0FP58 and A0A0L0FLW4) and *Capsaspora owczarzaki*, (A0A0D2WLY6 and A0A0D2UG33)—encode fungal AC homologs. The ACs from *F. alba* and *C. owczarzaki* contain four domains of an RA, an LRR, an ACD, and a PP2C, while *S. arctica* AC lacks an RA among those four. *F. alba* AC with N = 23 has no IR. However, in *S. arctica* AC with N = 18 and *C. owczarzaki* ACs with N = 22 and 21, IRs are identified at the corresponding position in the LRR domains in fungal ACs. The IR in *S. arctica* AC is 186-residue long. The IRs in two C. *owczarzaki* ACs are 328 and 28 residues long. The long IRs are IDRs, as seen in many fungal ACs.

An Arabidopsis thaliana LRR protein (At3g14460) acts as an adenylyl cyclase [95]. This protein contains an NB-ARC domain, an Rx N-terminal domain and a disease resistance protein Winged helix domain. This LRR domain with N = 30 has a 178 - residue IR which is positioned at the central part between *LRR16* and *LRR17*. This long IR is an IDR.

6. Perspectives

The solved structures of the IR-containing LRR domains adopt a single solenoid structure or two solenoids. Transmembrane kinases (TMKs) are members of the plant LRR-LRK family. Both TMK1 and TMK3 contain thirteen LRRs having non-LRR IRs between the tenth and eleventh units [96]. The LRR domains adopt two solenoids [96]. The predicted structures of the AC LRR domains by AlphaFold2 form a single solenoid regardless of the IR length. To verify this hypothesis, we propose the characterization of the entire region of AC LRR domain with long IR in some fungal species and its spectroscopic experiments including CD or NMR, small angle X-ray scattering in solution. Finally, x-ray crystal diffraction of AC LRR domain or cryo-electron microscopy of AC are desired.

This review may bring up the next question. Are the characteristics of AC LRR domains associated with

the lifestyle of fungi (such as saprophytic/parasitic, aquatic/terrestrial), host adaptability, and virulence? For example, are the multiple IRs in the phylum *Chytridiomycota* related to aquatic traits, and is the intrinsically disordered feature of long IRs related to the environmental adaptability of fungi? Whether the multiple IRs in *Chytridiomycota* reflect ancestral traits or adaptations to aquatic environments remains to be investigated. The AC LRR domains are seen in not only fungal ACs but also ACs from Opisthokonta and plants. The study of the evolutionary origin of the IRs and their biological roles is significant.

7. Summary and Conclusion

This review indicates that over 99% of individual LRR domains in the Class III ACs of fungi consist of twenty-three repeats with an additional parallel β-strand at the C-terminus. The RUL is mainly 23 or 24 residues. The most distinctive feature is the presence of IR interrupting the LRRs between the 16th and the 17th units. The IRs may be roughly classified in two groups. The first group IRs are 10–50 residues long. The second group IRs are 50–220 residues long which are mostly intrinsically disordered regions. The long IRs are identified in many fungal ACs. The AC LRR domains adopt typical solenoid structures. The IRs form bulges or loops in non-globular state. Two possibilities are discussed for the role of IRs in AC LRR domains. We propose a new subclass (IIId) of the class III ACs of fungi which includes an FHA.

Author Contributions

NM contributed to the concept, design, acquisition, analysis, interpretation, drafting of the manuscript, and final approval of the submitted document, and was accountable for the accuracy of this investigation. DB contributed to analysis. NM and DB contributed to editorial changes in the manuscript. Both authors have read and agreed to the published version of the manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflicts of Interest

The authors declare no conflicts of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/FBL50167.

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