Armadillo repeat proteins: beyond the animal kingdom

Coates, Juliet

DOI:
10.1016/S0962-8924(03)00167-3

Document Version
Early version, also known as pre-print

Citation for published version (Harvard):
Coates, J 2003, 'Armadillo repeat proteins: beyond the animal kingdom', *Trends in Cell Biology*, vol. 13, no. 9, pp. 463-471. https://doi.org/10.1016/S0962-8924(03)00167-3

Armadillo repeat proteins: beyond the animal kingdom

Juliet C. Coates

Juliet C. Coates

Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge, UK CB2 3EA.

Corresponding author: **Juliet C. Coates (jcc44@cam.ac.uk)**

Armadillo (Arm) repeat proteins contain tandem copies of a degenerate protein sequence motif that forms a conserved three-dimensional structure. Animal Arm repeat proteins function in various processes, including intracellular signalling and cytoskeletal regulation. A subset of these proteins are conserved across eukaryotic kingdoms, and non-metazoa such as *Dictyostelium* and *Chlamydomonas* possess homologues of members of the animal Arm repeat family. Higher plants also possess Arm repeat proteins, which, like their animal counterparts, function in intracellular signalling. Notably, these plant Arm proteins have novel functions. In addition, genome sequencing has identified a plethora of Arm-related proteins in *Arabidopsis*.

Proteins containing Arm repeats possess tandem imperfect repeats of a sequence motif of about 42 amino acids [1,2]. The 'Armadillo' nomenclature originates from the appearance of embryos that are mutant for the *Drosophila* segment polarity gene *armadillo*, the founding member of the family [3,4]. *Drosophila* Arm is the homologue of mammalian β-catenin, which is required both for cell–cell adhesion and for regulating gene expression during development (reviewed in ref. [5]). Many other proteins also contain Arm repeats, and all of these are thought to share a conserved three-dimensional structure (Fig. 1). A single Arm repeat consists of three α helices [2]. Tandem Arm repeats fold together and interact extensively with one another to form a right-handed superhelix of helices, which creates a surface for protein–protein interactions [2,6–8] (Fig. 1). Arm repeat proteins are structurally related to proteins containing tandem HEAT motifs, and the two protein families probably had a common phylogenetic origin [9].

Although originally characterized in animals, proteins containing Arm repeats also exist outside the animal kingdom. Arm family proteins with known functions can be divided into several categories, with different subfamilies having characteristic sequences outside the Arm domain that contribute to protein function (Fig. 2). Searches of the Pfam (http://www.sanger.ac.uk/Software/Pfam) and MATDB (http://mips.gsf.de/proj/thal/index.html) databases, as well as BLAST searches [10] using sequences of known Arm proteins, have detected at least 80 putative proteins containing tandem Arm repeats in *Arabidopsis* (Fig. 3), some of which have relatives in other plant species. As in other organisms, plant proteins that contain Arm repeats can be divided into several subfamilies (Fig. 3).

The presence of Arm repeat proteins in unicellular eukaryotes, animals and plants suggests that this protein family has ancient evolutionary origins. One hypothesis is that Arm family proteins have functions in plants similar to those in other organisms, and several recent studies have shown that this suggestion is partly correct. But plant Arm family proteins have been also discovered to have novel functions that correlate with the presence of plant-specific functional groups adjacent to the Arm repeat domain.

In this review, I outline the known functions of Arm repeat proteins across kingdoms and speculate on the functions of the numerous uncharacterized Arm family members detected in the completed *Arabidopsis* genome sequence.

Importin-α homologues are conserved in all eukaryotes

Importin-α homologues are conserved across eukaryotic kingdoms (Fig. 2). The function of importin-α is to regulate, through nuclear pore complexes, the transport of proteins into the nucleus (reviewed in ref. [11]). Proteins containing nuclear localization signals (NLSs) are recognized by the Arm repeats of importin-α (reviewed in ref. [12]). The amino (N)-terminus (non-Arm) region of importin-α binds to importin-β, which contains HEAT motifs [13]. Importin-β interacts with the nuclear pore, allowing translocation of the importin-α/importin-β heterodimer and its NLS-containing cargo into the nucleus, where the cargo is then released by the GTP-bound form of the small GTPase Ran (reviewed in refs [11,12]).

Notably, importin-α can interact with cytoskeletal components, suggesting a way in which nuclear import might be regulated. Immunolocalization studies have demonstrated that importin-α colocalizes with both microtubules and actin microfilaments in tobacco protoplasts [14], and that both tobacco and *Drosophila* importin-α associate with the cytoskeleton, but only when bound to an NLS [14,15]. In addition, the *Saccharomyces cerevisiae* importin-α, Srp1p, associates with the actin-related protein Act2p, which is required for nuclear pore structure and function [16].

Unicellular Arm family proteins and their homologues

In addition to Srp1p, a second protein with Arm repeats exists in *S. cerevisiae*. Vac8p forms a link between intracellular membranes and the actin cytoskeleton. Vac8p co-sediments with filamentous actin and is associated with the vacuole membrane through the lipid modification of residues located N-terminal to the Arm domain [17,18]. *vac8* mutants cannot target new vacuolar membrane to the bud site during cell division [17–19]. Vac8p is also a component of inter-organelle junctions between the nuclear membrane and the vacuole, where it binds to and is required for the localization of the integral membrane protein Nvj1p [20]. In addition, Vac8p is

required for the fusion of vacuolar membranes within the cell [21,22].

Nd9p, an Arm repeat protein related to Vac8p, exists in the protozoan *Paramecium* and is required for regulated exocxytic membrane fusion between the trichocyst and plasma membranes [23]. As in Vac8p, the Arm repeat region in Nd9p is thought to be involved in protein–protein interactions, whereas the membrane interaction region lies outside the repeats [23]. Whether Nd9p interacts with the actin cytoskeleton is unknown.

Recent data suggest that the regulation of the microtubule cytoskeleton by an Arm family protein is conserved among organisms as divergent as unicellular algae and mammals. The unicellular alga *Chlamydomonas* requires an Arm repeat protein, PF16, for cellular motility brought about by the flagellum [24,25]. The PF16 protein contains eight Arm repeats and localizes along the length of the C1 microtubule – one of the central pair of microtubules in the nine-plus-two arrangement of microtubules found in the flagellum. PF16 is required specifically for the stability of this single type of microtubule [24,25]. Deletion analysis of PF16 has shown that all eight Arm repeats are required for its function and assembly into the flagellum, indicating that the Arm repeats form a single functional unit [24].

The mammalian homologue of PF16 is a testis-specific sperm protein, SPAG6, that interacts with tubulin *in vitro* and colocalizes with microtubules *in vivo* [26,27]. SPAG6 interacts specifically with the central pair of microtubules in sperm tails, in a manner strikingly similar to that of PF16 [28]. Male mice lacking the *Spag6* gene are infertile owing to a sperm motility defect, and many *Spag6*$^{-/-}$ sperm lack the central pair of microtubules in the flagellum [28]. An interaction partner of SPAG6 is PF20, a WD40 repeat protein that is also localized to the central apparatus of sperm tails. PF20 requires interaction with SPAG6 for its localization to microtubules when both proteins are heterologously expressed in COS cells [29]. *Chlamydomonas* PF20 localizes to the C2 microtubule in the central pair, and *pf20* mutants lack the central pair of microtubules from the flagellum [30]. Thus, both the function and the interaction partners of PF16 and SPAG6 seem to have arisen early in evolution.

Other Arm family proteins that interact with microtubules

Although no obvious homologues of PF16 and SPAG6 seem to exist in higher plants, *Arabidopsis* has three Arm repeat proteins that are highly likely to interact with microtubules, because they possess an N-terminal kinesin domain [31] (Fig. 3). Known plant kinesins have roles in morphogenesis and development affecting cell division and cell growth (reviewed in refs [32,33]). Presumably, the Arm repeat domain of the *Arabidopsis* kinesin proteins interacts with target proteins, thereby localizing them to microtubules.

It is possible that *Arabidopsis* kinesin Arm proteins function analogously to a complex of Arm repeat proteins that interacts with microtubules in mammalian cells. Mammalian kinesin family proteins interact with an Arm family protein called SMAP (Smg-GDS-associated protein), which in turn interacts with Smg-GDS, an Arm repeat exchange factor for small GTPases [34,35] (Fig. 2). In addition, the adenomatous polyposis coli (APC) protein can, through its own Arm repeats, interact with SMAP, and this interaction is required for microtubule clustering [36]. Notably, a null mutant in the *Dictyostelium* Smg-GDS homologue is defective in chemotaxis and aggregation, although the mechanism underlying this defect is unclear [37].

Cytoskeletal functions of β-catenin and related proteins

The first Arm repeat protein to be implicated in cytoskeletal regulation in multicellular organisms was β-catenin, a component of adherens junctions in animals. Adherens junctions are points of intercellular contact, where cadherin molecules at the plasma membrane are anchored to the actin cytoskeleton via the adaptor proteins α- and β-catenin [38]. β-Catenin binds directly to the intracellular tail of cadherin through its Arm repeats [39]; the N-terminus of β-catenin in turn interacts with α-catenin [40], which interacts with actin [41]. Desmosomes – specialized adhesive structures that are prevalent in tissue types such as epithelia and link keratin intermediate filaments to desmosomal cadherins – contain plakoglobin, a close relative of β-catenin [42,43]. It has been shown that intact adherens junctions containing Arm, the *Drosophila* homologue of β-catenin, are necessary for cellular rearrangements that take place during morphogenesis [44]. A similar requirement is found for HMP-2, a β-catenin homologue in *Caenorhabditis elegans* [45].

Animals possess other Arm repeat proteins with a cytoskeletal role similar to that of β-catenin (Fig. 2). Adherens junctions also contain p120 protein and its relatives (δ-catenin, plakophilins and p0071) [46]. Desmosomes also contain members of the p120 subfamily [46]. p120 can affect the activity of Rho family GTPases, which might regulate the balance between cell motility and adhesion, via the actin cytoskeleton (reviewed in ref. [47]). The p120 family of proteins seems to be restricted to vertebrates and arthropods, suggesting that this subgroup might have evolved coordinately with the diversification of specialized types of tissue in complex animals.

The cytoskeletal function of β-catenin is conserved in a multicellular non-metazoan (Fig. 2). The *Dictyostelium* β-catenin-related protein Aardvark (Aar) localizes to and is necessary for the integrity of actin-containing adherens junctions during the formation of fruiting bodies [48,49]. It is not known, however, whether Aar interacts with a cadherin protein, because the only cadherin-related protein of known function in *Dictyostelium*, DdCad-1, lacks a transmembrane domain and thus a conserved region for interaction with β-catenin [50]. The fact that β-catenin-like proteins exist both in *Dictyostelium* and in metazoa, and that Arm proteins that interact with the cytoskeleton are present in unicellular organisms, raises the possibility that Arm repeat proteins have a conserved cytoskeletal function in all organisms, and that proteins with similar functions also exist in higher plants.

Plant cells are surrounded by a rigid cell wall and so do not possess 'classical adherens junctions' linking one cell membrane directly to another. Indeed, plants do not seem

to possess any close relatives of metazoan adherens junction proteins other than Arm repeat proteins. However, adjacent plant cells are linked via membrane-lined channels, plasmodesmata, which also contain actin filaments (reviewed in ref. [51]). As yet, it has not been ascertained to what extent plasmodesmata share molecular similarities with animal junctions, but it is tempting to speculate that some plant Arm repeat proteins might turn out to be plasmodesmatal components.

β-Catenin and Wnt signal transduction

In addition to their cytoskeletal functions, β-catenin and its homologues act as regulators of gene expression both during development and throughout adult life. These proteins can enter the nucleus in response to extracellular signals and bind to DNA in a complex with T-cell factor (TCF) transcription factors, thus altering gene expression (reviewed in ref. [52]).

In essence, cytosolic β-catenin or Arm can be stabilized by extracellular glycoprotein signals, known as Wnts in animals, that are transduced by a seven-transmembrane receptor. This stabilization involves preventing glycogen synthase kinase-3 (GSK-3) from phosphorylating β-catenin on N-terminal serines and threonines [53]. In the absence of Wnt signals, phosphorylation of β-catenin targets it for degradation by the proteasome via interaction with a protein complex that includes the F-box/WD40 repeat protein β-Trcp (Slimb in *Drosophila*), as well as GSK-3, Axin and APC [52]. By contrast, stabilized β-catenin is targeted to the nucleus, where it can affect gene expression. β-Catenin and TCF transcriptional targets include regulators of cell proliferation, transcription factors required during development, and cell adhesion molecules [52].

Arm repeat proteins have not been shown to have a function in gene expression or signalling in unicellular organisms but do have a conserved signalling function in *Dictyostelium*. The *Dictyostelium* β-catenin homologue Aar is required for the differentiation of prespore and spore cell types, and for expression of the prespore cell-specific gene *psA* [48]. As in animals, the signalling function of Aar is dependent on *Dictyostelium* GSK-3 [48]; however, it is not known whether Aar interacts with TCF-like proteins during this process. Notably, Aar is more similar to proteins in *Arabidopsis* and *Oryza* than to its metazoan β-catenin counterparts, both in sequence similarity (by BLAST) and due to an F-box in Aar and in the plant proteins that is not present in metazoan β-catenin (Box 1 and see below).

Other metazoan Arm repeat proteins might also have a dual cytoskeletal and transcriptional role. Mammalian p120, its relatives and the more distantly related plakophilins are all detected in cell nuclei, and the nuclear localization of p120 is regulated by the generation of alternatively spliced p120 isoforms with or without a nuclear export signal [54]. In addition, p120 interacts with Kaiso, a poxvirus and zinc-finger (POZ) transcription factor [55], which inhibits the interaction between Kaiso and DNA [56]. Of interest, *Arabidopsis* possesses a protein (At5g13060) that comprises Arm repeats and a 'Broad-complex Tramtrack Bric-a-brac' (BTB)/POZ DNA-binding domain (Fig. 3). Perhaps this protein has an analogous transcriptional role to that of the p120–Kaiso complex found in mammalian cells. Similarly, *Arabidopsis* At1g08320 contains a basic zipper (bZIP) domain in addition to Arm repeats (Fig. 3). bZIP domains mediate both protein dimerization and transcriptional regulation, suggesting that At1g08320 is also likely to be a transcription factor or part of a transcriptional complex.

PHOTOPERIOD RESPONSIVE 1 functions in light and gibberellin signalling

Recent work by Amador *et al.* [57] is the first to demonstrate the movement of Arm repeat plant proteins into the nucleus in response to extracellular signals. The PHOTOPERIOD RESPONSIVE 1 (PHOR1) protein of potato (*Solanum tuberosum*) possesses seven Arm repeats located downstream from a U-box motif (also referred to as a CPI domain [57]). U-box proteins, so called because of their homology to *S. cerevisiae* UFD2, are predicted to be components of the cellular ubiquitination machinery, which targets proteins for proteolytic degradation (reviewed in refs [58,59]).

PHOR1 mRNA is upregulated under short-day conditions, which are required for potato tuberization. *PHOR1* mRNA also undergoes diurnal variation, with levels increasing after the transition to light. In short days, a second peak in *PHOR1* mRNA expression is seen at dusk [57]. Inhibition of *PHOR1* through the expression of an antisense construct results in early and increased tuberization under short-day conditions and also produces shorter stem height [57].

PHOR1 functions in the gibberellin (GA) hormone signalling pathway. *PHOR1* antisense lines are less sensitive than wild-type plants to exogenously applied GA, as measured by both stem elongation and the expression of GA target genes, whereas plants overexpressing *PHOR1* show an enhanced response to GA application and reduced sensitivity to an inhibitor of GA biosynthesis [57]. In transformed tobacco cells, a fusion protein of PHOR1 and green fluorescent protein (GFP) is present in both the nucleus and the cytosol. Notably, the distribution of PHOR1–GFP changes in response to GA. PHOR1–GFP translocates to the nucleus transiently in response to exogenous GA, and is predominantly cytosolic in cells treated with an inhibitor of GA biosynthesis [57]. Deletion analysis of PHOR1 domains has shown that nuclear targeting of PHOR1–GFP requires the Arm repeat domain, whereas cytosolic localization seems to be mediated by the U-box motif [57].

ARC1 signalling in the *Brassica* self-incompatibility response

A protein related to PHOR1, Arm repeat containing 1 (ARC1), has been characterized in *Brassica* [60]. ARC1 was identified as a specific binding protein for the kinase domain of *Brassica* S-locus receptor kinases (SRKs). SRKs are transmembrane serine/threonine kinases required for the self-incompatibility (SI) response that prevents a plant self-fertilizing. *arc-1* is expressed specifically in the stigma, the female part of the plant to which pollen, containing the male gametes, binds [60]. *In vitro* binding of ARC1 to SRKs is dependent on phosphorylation, and

ARC1 is also phosphorylated by the SRKs themselves [60]. Inhibition of ARC1 through expression of an antisense construct results in a partial loss of SI [61], which suggests that it is a positive regulator of the SI response. ARC1 can enter the nucleus and contains functional nuclear localization and nuclear export signals [62]. Like PHOR1, ARC1 contains a U-box.

As mentioned above, U-box proteins are thought to target proteins for degradation [58,59]. Recent work has shown that ARC1 functions *in vitro* as an E3 ubiquitin ligase that can interact both with the ubiquitination machinery and with target proteins that become ubiquitinated [62]. This E3 ligase activity requires the U-box and is also likely to function *in vivo*. ARC1 protein colocalizes with the proteasome in tobacco cells cotransformed with active SRK; this colocalization requires the ARC1 U-box.

Brassica pistils (comprising the stigmas and associated female structures) pollinated with self-incompatible pollen have increased levels of ubiquitinated proteins as compared with non-pollinated or compatibly pollinated controls. This increase in ubiquitination, however, is lost in plants expressing an ARC1 antisense construct. In addition, treating pistils with proteasome inhibitors blocks the SI response, allowing self-incompatible pollen to germinate and to grow [62]. Thus, the apparent function of ARC1 in the SI response is to target several proteins for degradation by the proteasome, specifically on activation of the SRK. It is likely that these target proteins, which usually would be required for aspects of fertilization, bind to the Arm repeats. ARC1 could shuttle some of its target proteins from the nucleus to the proteasome, or it might have an additional, independent nuclear function.

Potential proteasomal functions for other plant Arm family proteins?

By far the largest Arm repeat protein subgroup in *Arabidopsis* is the one containing relatives of PHOR1 and ARC1, which have a predicted U-box upstream of the Arm domain. Some relatives were identified by Azevedo *et al.* [58,59] and some by Amador *et al.* [57], but searches of the *Arabidopsis* proteome by BLAST and Pfam suggest that *Arabidopsis* possesses at least 35 PHOR1- and ARC1-related proteins (Fig. 3). By analogy to ARC1 and PHOR1, it is likely that *Arabidopsis* U-box proteins have diverse functions and interaction partners, but at least some might enter the nucleus in response to extracellular signals or mediate the regulated degradation of target proteins.

In addition to the large family of proteins with Arm repeats and a U-box, a few *Arabidopsis* Arm proteins contain other domains present in proteins that interact with the ubiquitination machinery [59]. At5g02880 possesses a predicted 'homologous to the E6-AP C-terminus' (HECT) domain (Fig. 3), and At2g44900 and At3g60350 both contain a predicted F-box – a motif of 40–50 amino acids that was originally identified in cyclin F [63–65]. Known proteins with HECT domains and F-boxes function as E3 ubiquitin ligases, which target interacting proteins for ubiquitination and subsequent degradation by the proteasome. Given that targeted proteasomal degradation of proteins seems to be integral to plant physiology and development, for example, in hormone signalling and responses to light (reviewed in ref. [59]), and because Arm repeats function as sites of protein–protein interaction, many *Arabidopsis* Arm proteins are likely to have important and diverse roles in targeted protein degradation throughout the life of the plant.

Potential 'Wnt-like' signalling functions for plant Arm family proteins?

It has been suggested that, other than GSK-3, there are no Wnt signalling pathway homologues in *Arabidopsis* [66,67]. But BLAST searches based on the *Dictyostelium* Aar protein sequence have identified two genes, At2g44900 and At3g60350, that encode proteins with the most similarity in structure and sequence to Aar, Arm or β-catenin of any *Arabidopsis* proteins (Box 1). These two proteins, for which I propose the names Arabidillo-1 and Arabidillo-2 (for '*Arabidopsis* Armadillo') consist of a core of at least nine Arm repeats, several putative sites for GSK-3 phosphorylation and an N-terminal F-box (Box 1) [64,65]. An apparent homologue of Arabidillo-1 and Arabidillo-2 also exists in *Oryza* (AAG60190).

The presence of a putative F-box in Aar, Arabidillo-1 and Arabidillo-2 suggests that these proteins might target other proteins for destruction by ubiquitin-mediated proteolysis. In addition, there is evidence that F-box-containing proteins can themselves be degraded autocatalytically when bound to the ubiquitination machinery in yeast, animal cells and plants (ref. [68] and references therein). In metazoa, phosphorylated β-catenin is targeted for destruction by β-Trcp, a protein that contains an F-box and WD40 repeats [69]. Aar, Arabidillo-1 and Arabidillo-2 all possess an integral F-box, which could perhaps allow them to be targeted to the proteasome directly in the absence of a stabilizing signal, bypassing the need for the more complex destruction mechanism found in animal systems. *Arabidopsis* does, however, also possess two F-box/WD40 repeat proteins, At3g52030 and At5g21040 [64,65,70]. It has yet to be determined whether Arabidillo-1 and Arabidillo-2 are regulated in a manner analogous to the regulation of β-catenin – that is, either by constitutive degradation in the absence of a stabilizing signal or by GSK-3 phosphorylation.

Arabidopsis possesses ten homologues of GSK-3. Some of these are expressed in a tissue-specific manner and some have known developmental functions – for example, in flower development and hormone signalling (reviewed in ref. [66]). In addition, the *Arabidopsis* GSK3 homologue BIN-2 (also known as UCU1 or DWF-12) regulates the stability and nuclear localization of BES-1 and BZR-1 proteins in response to brassinosteroid signals, in a similar manner to the regulation of β-catenin homologues by Wnt signalling [71–73]. Although BES-1 and BZR-1 are not members of the Arm repeat family, it remains to be determined whether plant Arm proteins can be regulated in the same way.

Concluding remarks

Members of the Arm repeat protein family are present in all eukaryotic kingdoms. The structure of the Arm repeat

domain allows these proteins to have many interaction partners and functions in the cell. Although most extensively studied in animals, some Arm family members clearly have non-animal homologues, with functions that have been conserved throughout evolution.

Recent studies have shown that plants possess many proteins containing Arm repeats. In *Arabidopsis*, the largest subgroup of Arm proteins is that of proteins containing an associated U-box – a motif associated with proteasomal functions. This U-box/Arm family seems to be unique to higher plants as there are no detectable counterparts in other genomes, and its large number of members implies great functional diversification. Two members of the U-box/Arm family have been characterized. Potato PHOR1 functions in both light and gibberellin signalling, and *Brassica* ARC1 is involved in the ubiquitination of target proteins during the SI response. Despite having novel functions, plant and non-plant Arm family members have similarities in their regulation – for example, their nuclear accumulation in response to extracellular signals and their regulation by phosphorylation.

With the large amount of genomic information now available, it will be possible to discover the role of the many uncharacterized Arm repeat proteins in various systems, for example, by investigating their intracellular localization and their loss-of-function phenotypes. Future studies of plant Arm repeat proteins are likely to provide insights into the mechanisms of many aspects of plant cell biology and development.

Acknowledgements

This work was supported by a Gatsby Charitable Foundation Postdoctoral Research Fellowship and was done in Jim Haseloff's laboratory. I thank Adrian Harwood, Jim Haseloff and Mark Tester for critically reading the manuscript.

References

1 Peifer, M. *et al.* (1994) A repeating amino acid motif shared by proteins with diverse cellular roles. *Cell* 76, 789–791

2 Huber, A.H. *et al.* (1997) Three-dimensional structure of the armadillo repeat region of β-catenin. *Cell* 90, 871–882

3 Nusslein-Volhard, C. and Wieschaus, E. (1980) Mutations affecting segment number and polarity in *Drosophila*. *Nature* 287, 795–801

4 Riggleman, B. *et al.* (1989) Molecular analysis of the *armadillo* locus: uniformly distributed transcripts and a protein with novel internal repeats are associated with a *Drosophila* segment polarity gene. *Genes Dev.* 3, 96–113

5 Conacci-Sorrell, M. *et al.* (2002) The cadherin–catenin adhesion system in signaling and cancer. *J. Clin. Invest.* 109, 987–991

6 Conti, E. *et al.* (1998) Crystallographic analysis of the recognition of a nuclear localization signal by the nuclear import factor karyopherin α. *Cell* 94, 193–204

7 Daniels, D.L. *et al.* (2001) β-Catenin: molecular plasticity and drug design. *Trends Biochem. Sci.* 26, 672–678

8 Conti, E. and Kuriyan, J. (2000) Crystallographic analysis of the specific yet versatile recognition of distinct nuclear localization signals by karyopherin α. *Struct. Fold. Des.* 8, 329–338

9 Andrade, M.A. *et al.* (2001) Comparison of ARM and HEAT protein repeats. *J. Mol. Biol.* 309, 1–18

10 Altschul, S.F. *et al.* (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25, 3389–3402

11 Gorlich, D. (1997) Nuclear protein import. *Curr. Opin. Cell Biol.* 9, 412–419

12 Conti, E. and Izaurralde, E. (2001) Nucleocytoplasmic transport enters the atomic age. *Curr. Opin. Cell Biol.* 13, 310–319

13 Malik, H.S. *et al.* (1997) Evolutionary specialization of the nuclear targeting apparatus. *Proc. Natl. Acad. Sci. U. S. A.* 94, 13738–13742

14 Smith, H.M. and Raikhel, N.V. (1998) Nuclear localization signal receptor importin α associates with the cytoskeleton. *Plant Cell* 10, 1791–1799

15 Gorjanacz, M. *et al.* (2002) Importin-α2 is critically required for the assembly of ring canals during *Drosophila* oogenesis. *Dev. Biol.* 251, 271–282

16 Yan, C. *et al.* (1997) A role for the divergent actin gene, ACT2, in nuclear pore structure and function. *EMBO J.* 16, 3572–3586

17 Pan, X. and Goldfarb, D.S. (1998) YEB3/VAC8 encodes a myristylated Armadillo protein of the *Saccharomyces cerevisiae* vacuolar membrane that functions in vacuole fusion and inheritance. *J. Cell Sci.* 111, 2137–2147

18 Wang, Y.X. *et al.* (1998) Vac8p, a vacuolar protein with armadillo repeats, functions in both vacuole inheritance and protein targeting from the cytoplasm to vacuole. *J. Cell Biol.* 140, 1063–1074

19 Fleckenstein, D. *et al.* (1998) Yel013p (Vac8p), an armadillo repeat protein related to plakoglobin and importin-α, is associated with the yeast vacuole membrane. *J. Cell Sci.* 111, 3109–3118

20 Pan, X. *et al.* (2000) Nucleus-vacuole junctions in *Saccharomyces cerevisiae* are formed through the direct interaction of Vac8p with Nvj1p. *Mol. Biol. Cell* 11, 2445–2457

21 Wang, Y.X. *et al.* (2001) Fusion of docked membranes requires the armadillo repeat protein Vac8p. *J. Biol. Chem.* 276, 35133–35140

22 Veit, M. *et al.* (2001) Vac8p release from the SNARE complex and its palmitoylation are coupled and essential for vacuole fusion. *EMBO J.* 20, 3145–3155

23 Froissard, M. *et al.* (2001) ND9P, a novel protein with armadillo-like repeats involved in exocytosis: physiological studies using allelic mutants in paramecium. *Genetics* 157, 611–620

24 Smith, E.F. and Lefebvre, P.A. (2000) Defining functional domains within PF16: a central apparatus component required for flagellar motility. *Cell Motil. Cytoskeleton* 46, 157–165

25 Smith, E.F. and Lefebvre, P.A. (1996) PF16 encodes a protein with armadillo repeats and localizes to a single microtubule of the central apparatus in *Chlamydomonas* flagella. *J. Cell Biol.* 132, 359–370

26 Neilson, L.I. *et al.* (1999) cDNA cloning and characterization of a human sperm antigen (SPAG6) with homology to the product of the *Chlamydomonas* PF16 locus. *Genomics* 60, 272–280

27 Sapiro, R. *et al.* (2000) Sperm antigen 6 is the murine homologue of the *Chlamydomonas reinhardtii* central apparatus protein encoded by the PF16 locus. *Biol. Reprod.* 62, 511–518

28 Sapiro, R. *et al.* (2002) Male infertility, impaired sperm motility, and hydrocephalus in mice deficient in sperm-associated antigen 6. *Mol. Cell. Biol.* 22, 6298–6305

29 Zhang, Z. *et al.* (2002) A sperm-associated WD repeat protein orthologous to *Chlamydomonas* PF20 associates with Spag6, the mammalian orthologue of *Chlamydomonas* PF16. *Mol. Cell. Biol.* 22, 7993–8004

30 Smith, E.F. and Lefebvre, P.A. (1997) PF20 gene product contains WD repeats and localizes to the intermicrotubule bridges in *Chlamydomonas* flagella. *Mol. Biol. Cell* 8, 455–467

31 Reddy, A.S. and Day, I.S. (2001) Kinesins in the *Arabidopsis* genome: a comparative analysis among eukaryotes. *BMC Genomics* 2, 2

32 Mayer, U. and Jurgens, G. (2002) Microtubule cytoskeleton: a track record. *Curr. Opin. Plant Biol.* 5, 494–501

33 Smith, L.G. (2003) Cytoskeletal control of plant cell shape: getting the fine points. *Curr. Opin. Plant Biol.* 6, 63–73

34 Shimizu, K. *et al.* (1996) SMAP, an Smg GDS-associating protein having arm repeats and phosphorylated by Src tyrosine kinase. *J. Biol. Chem.* 271, 27013–27017

35 Wedaman, K.P. *et al.* (1996) Sequence and submolecular localization of the 115kD accessory subunit of the

heterotrimeric kinesin-II (KRP85/95) complex. *J. Cell Biol.* 132, 371–380

36 Jimbo, T. *et al.* (2002) Identification of a link between the tumour suppressor APC and the kinesin superfamily. *Nat. Cell Biol.* 4, 323–327

37 Vithalani, K.K. *et al.* (1998) Identification of darlin, a *Dictyostelium* protein with Armadillo-like repeats that binds to small GTPases and is important for the proper aggregation of developing cells. *Mol. Biol. Cell* 9, 3095–3106

38 Ozawa, M. *et al.* (1989) The cytoplasmic domain of the cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species. *EMBO J.* 8, 1711–1717

39 Aberle, H. *et al.* (1994) Assembly of the cadherin–catenin complex *in vitro* with recombinant proteins. *J. Cell Sci.* 107, 3655–3663

40 Aberle, H. *et al.* (1996) Single amino acid substitutions in proteins of the armadillo gene family abolish their binding to α-catenin. *J. Biol. Chem.* 271, 1520–1526

41 Rimm, D.L. *et al.* (1995) α1(E)-catenin is an actin-binding and -bundling protein mediating the attachment of F-actin to the membrane adhesion complex. *Proc. Natl. Acad. Sci. U. S. A.* 92, 8813–8817

42 Cowin, P. *et al.* (1986) Plakoglobin: a protein common to different kinds of intercellular adhering junctions. *Cell* 46, 1063–1073

43 Franke, W.W. *et al.* (1989) Molecular cloning and amino acid sequence of human plakoglobin, the common junctional plaque protein. *Proc. Natl. Acad. Sci. U. S. A.* 86, 4027–4031

44 Cox, R.T. *et al.* (1996) Armadillo is required for adherens junction assembly, cell polarity, and morphogenesis during *Drosophila* embryogenesis. *J. Cell Biol.* 134, 133–148

45 Costa, M. *et al.* (1998) A putative catenin–cadherin system mediates morphogenesis of the *Caenorhabditis elegans* embryo. *J. Cell Biol.* 141, 297–308

46 Anastasiadis, P.Z. and Reynolds, A.B. (2000) The p120 catenin family: complex roles in adhesion, signaling and cancer. *J. Cell Sci.* 113, 1319–1334

47 Anastasiadis, P.Z. and Reynolds, A.B. (2001) Regulation of Rho GTPases by p120-catenin. *Curr. Opin. Cell Biol.* 13, 604–610

48 Grimson, M.J. *et al.* (2000) Adherens junctions and β-catenin-mediated cell signalling in a non-metazoan organism. *Nature* 408, 727–731

49 Coates, J.C. *et al.* (2002) Loss of the β-catenin homologue Aardvark causes ectopic stalk formation in *Dictyostelium*. *Mech. Dev.* 116, 117–127

50 Wong, E.F.S. *et al.* (1996) Molecular cloning and characterization of DdCAD-1, a Ca^{2+}-dependent cell–cell adhesion molecule, in *Dictyostelium discoideum*. *J. Biol. Chem.* 271, 16399–16408

51 Heinlein, M. (2002) Plasmodesmata: dynamic regulation and role in macromolecular cell-to-cell signaling. *Curr. Opin. Plant Biol.* 5, 543–552

52 Behrens, J. (2000) Control of β-catenin signaling in tumor development. *Ann. N. Y. Acad. Sci.* 910, 21–33

53 van Noort, M. *et al.* (2002) Wnt signaling controls the phosphorylation status of β-catenin. *J. Biol. Chem.* 277, 17901–17905

54 van Hengel, J. *et al.* (1999) Nuclear localization of the p120(ctn) Armadillo-like catenin is counteracted by a nuclear export signal and by E-cadherin expression. *Proc. Natl. Acad. Sci. U. S. A.* 96, 7980–7985

55 Daniel, J.M. and Reynolds, A.B. (1999) The catenin p120(ctn) interacts with Kaiso, a novel BTB/POZ domain zinc finger transcription factor. *Mol. Cell. Biol.* 19, 3614–3623

56 Daniel, J.M. *et al.* (2002) The p120(ctn)-binding partner Kaiso is a bi-modal DNA-binding protein that recognizes both a sequence-specific consensus and methylated CpG dinucleotides. *Nucleic Acids Res.* 30, 2911–2919

57 Amador, V. *et al.* (2001) Gibberellins signal nuclear import of PHOR1, a photoperiod-responsive protein with homology to *Drosophila* armadillo. *Cell* 106, 343–354

58 Azevedo, C. *et al.* (2001) The U-box protein family in plants. *Trends Plant Sci.* 6, 354–358

59 Vierstra, R.D. (2003) The ubiquitin/26S proteasome pathway, the complex last chapter in the life of many plant proteins. *Trends Plant Sci.* 8, 135–142

60 Gu, T. *et al.* (1998) Binding of an arm repeat protein to the kinase domain of the S-locus receptor kinase. *Proc. Natl. Acad. Sci. U. S. A.* 95, 382–387

61 Stone, S.L. *et al.* (1999) A breakdown of *Brassica* self-incompatibility in ARC1 antisense transgenic plants. *Science* 286, 1729–1731

62 Stone, S.L. *et al.* (2003) ARC1 is an E3 ubiquitin ligase and promotes the ubiquitination of proteins during the rejection of self-incompatible *Brassica* pollen. *Plant Cell* 15, 885–898

63 Bai, C. *et al.* (1996) SKP1 connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-box. *Cell* 86, 263–274

64 Gagne, J.M. *et al.* (2002) The F-box subunit of the SCF E3 complex is encoded by a diverse superfamily of genes in *Arabidopsis*. *Proc. Natl. Acad. Sci. U. S. A.* 99, 11519–11524

65 Kuroda, H. *et al.* (2002) Classification and expression analysis of *Arabidopsis* F-box-containing protein genes. *Plant Cell Physiol.* 43, 1073–1085

66 Jonak, C. and Hirt, H. (2002) Glycogen synthase kinase 3/SHAGGY-like kinases in plants: an emerging family with novel functions. *Trends Plant Sci.* 7, 457–461

67 Wigge, P.A. and Weigel, D. (2001) *Arabidopsis* genome: life without notch. *Curr. Biol.* 11, R112–R114

68 Kim, W.Y. *et al.* (2003) Circadian phase-specific degradation of the F-box protein ZTL is mediated by the proteasome. *Proc. Natl. Acad. Sci. U. S. A.* 100, 4933–4938

69 Jiang, J. and Struhl, G. (1998) Regulation of the Hedgehog and Wingless signalling pathways by the F-box/WD40-repeat protein Slimb. *Nature* 391, 493–496

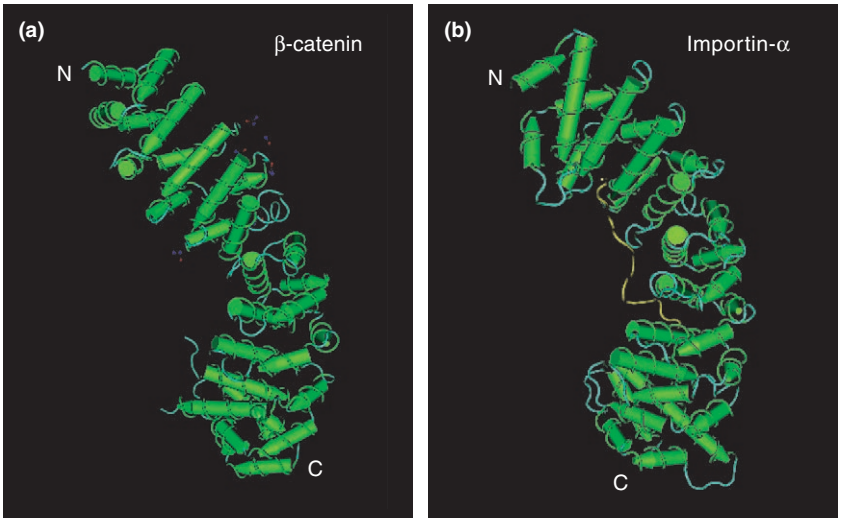
70 Xiao, W. and Jang, J. (2000) F-box proteins in *Arabidopsis*. *Trends Plant Sci.* 5, 454–457

71 He, J.X. *et al.* (2002) The GSK3-like kinase BIN2 phosphorylates and destabilizes BZR1, a positive regulator of the brassinosteroid signaling pathway in *Arabidopsis*. *Proc. Natl. Acad. Sci. U. S. A.* 99, 10185–10190

72 Yin, Y. *et al.* (2002) BES1 accumulates in the nucleus in response to brassinosteroids to regulate gene expression and promote stem elongation. *Cell* 109, 181–191

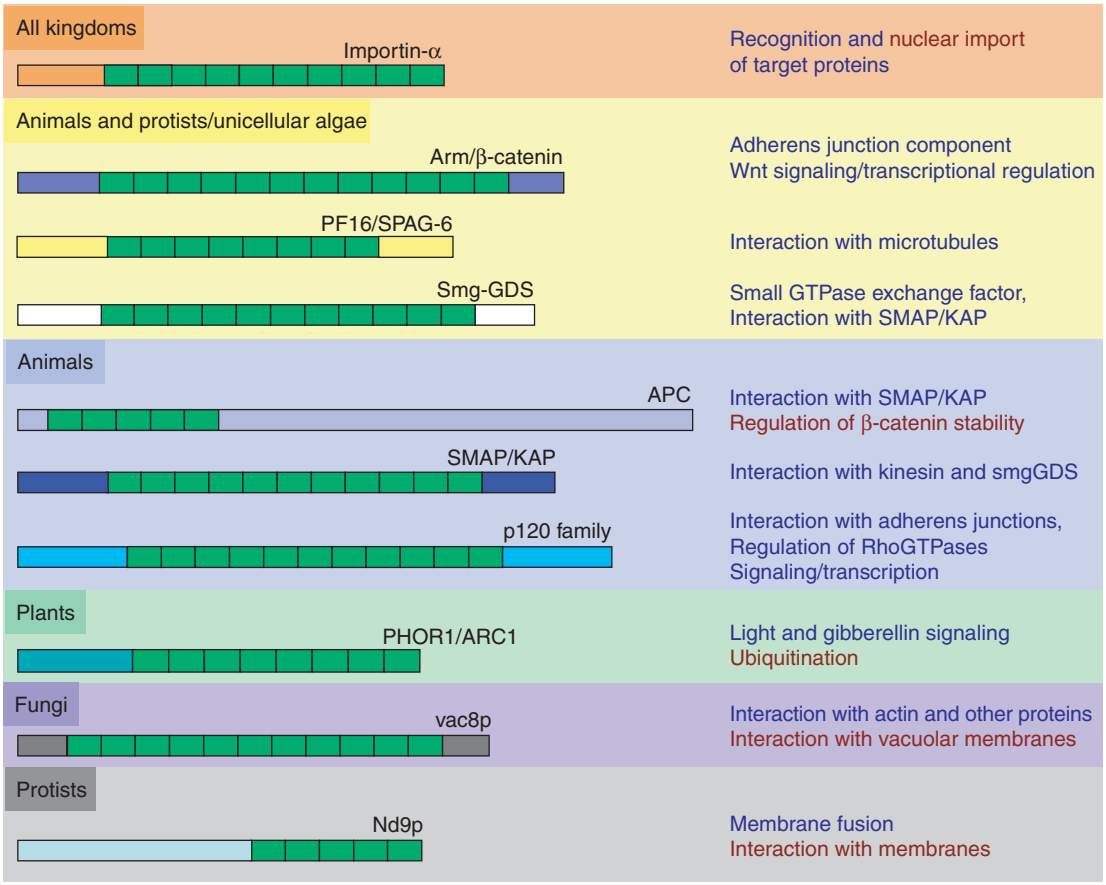
73 Zhao, J. *et al.* (2002) Two putative BIN2 substrates are nuclear components of brassinosteroid signaling. *Plant Physiol.* 130, 1221–1229

74 Thompson, J.D. *et al.* (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22, 4673–4680



TRENDS in Cell Biology

Fig. 1. Arm repeat proteins have a conserved three-dimensional structure. Three-dimensional structures of the Arm repeat regions of mouse β-catenin (left) and yeast (*S. cerevisiae*) importin-α (right). Both proteins form a very similar helical bundle of α helices, which are depicted as green cylinders. Importin-α is shown in a complex with the nuclear localization signal (NLS) peptide from *Xenopus* Nucleoplasmin (yellow strand), which fits into the groove formed by the superhelix of helices. Figures were generated using Cn3D software (http://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml) from the structures 3BCT (β-catenin, 457 amino acids [2]) and 1EE5 (importin-α, 424 amino acids [8]) in the Protein Data Bank (http://www.rcsb.org/pdb/). The N- and C-terminus of each Arm repeat region is marked.



TRENDS in Cell Biology

Fig. 2. Conservation of Arm repeat proteins in eukaryotes. Shown are the subfamilies of Arm repeat proteins with known functions. Green boxes indicate Arm repeats, other coloured boxes represent sequences specific to each protein subfamily. Proteins are drawn roughly to scale apart from APC, which varies from 1067 to 2845 amino acids among species. Each Arm repeat is about 42 amino acids. A brief description of the function of each protein is given on the right: functions known to be not associated with the Arm domain are listed in red; those associated with the Arm domain are listed in blue. Proteins are arranged according to their presence in various eukaryotic kingdoms: orange background, proteins found in all kingdoms; yellow background, proteins found in both animals and protists or unicellular algae; blue background, proteins found only in animals; light green background, proteins found only in plants; mauve background, proteins found only in fungi; grey background, proteins found only in protists. Although no function for importin-α in protists has been reported, there is a distinct homologue of importin-α in *Dictyostelium* (EMBL accession code AC116030), which has been included to illustrate the conservation of this protein across all eukaryotic kingdoms.

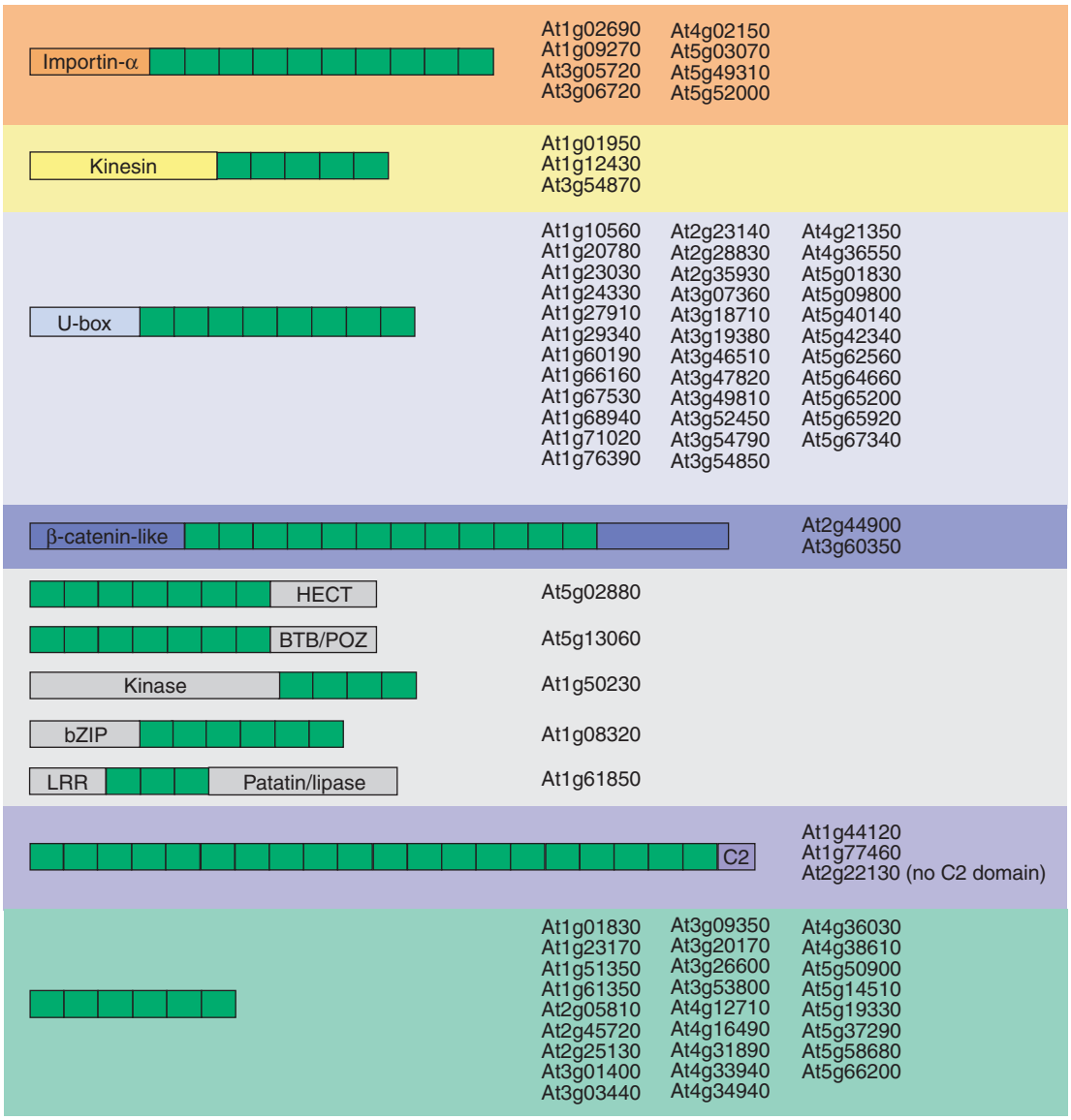
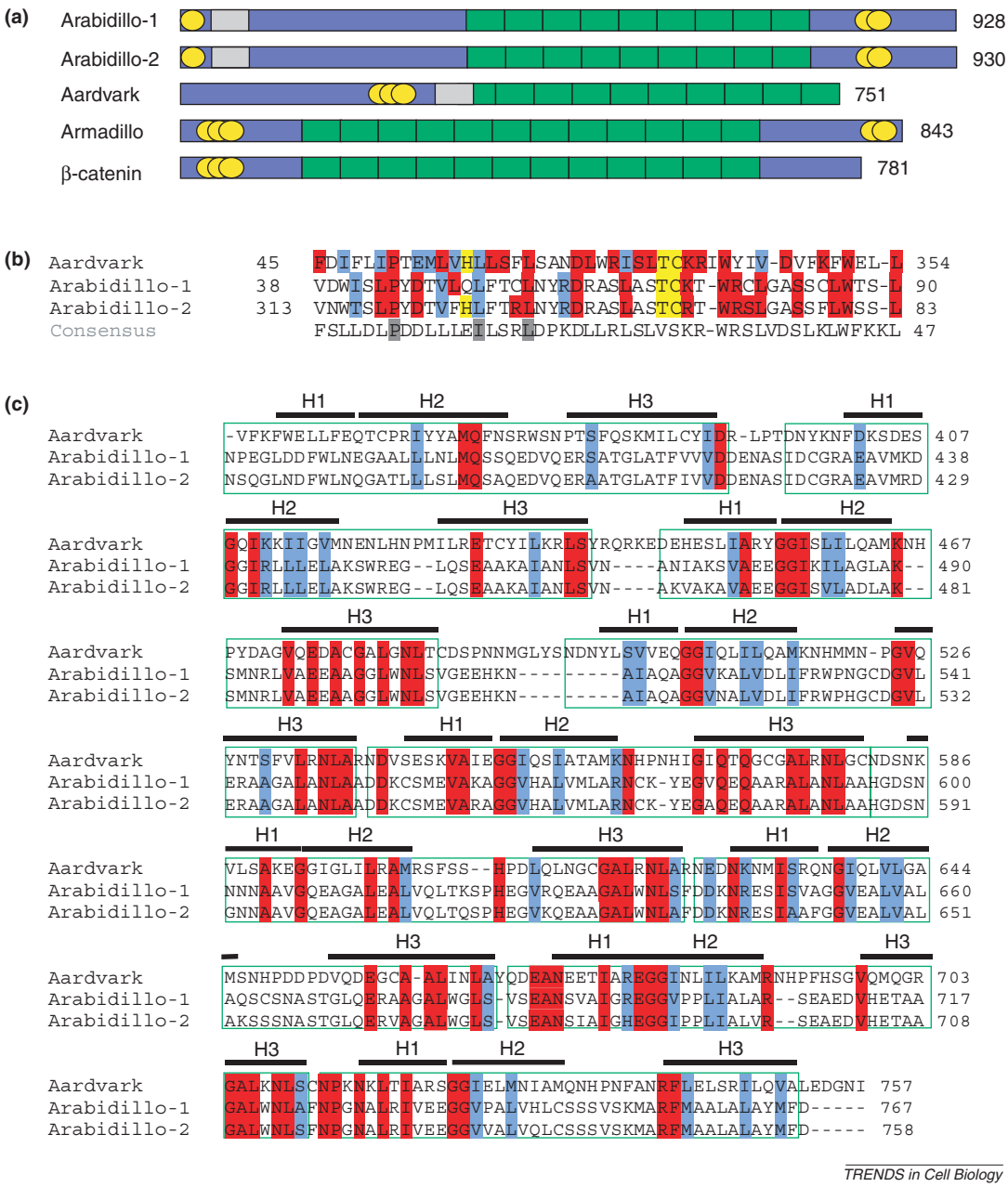


Fig. 3. Arm repeat proteins in *Arabidopsis*. Putative genes encoding proteins with tandem Arm repeats have been compiled from the *Arabidopsis* genome. *Arabidopsis* Arm repeat proteins can be subdivided on the basis of their homology with each other and with proteins from other organisms. Orange background, importin-α family; yellow background, proteins with a kinesin domain; light blue background, predicted U-box-containing proteins similar to PHOR1/ARC1; dark blue background, proteins with similarity to Arm, β-catenin or Aar; grey background, proteins containing a predicted motif in addition to the Arm repeats – namely, a HECT domain (Pfam PF00632), a BTB/POZ domain (PF00651), a serine/threonine kinase domain (PF00069), a bZIP domain (PF00170), a leucine-rich repeat (LRR) domain (PF00560) and a patatin-like phospholipase domain (PF01734); mauve background, proteins with about 20 Arm repeats (two of which also have a C-terminal C2 domain); light green background, proteins containing Arm repeats but no other recognizable protein motifs. In the different categories of protein, green boxes indicate Arm repeats and other coloured boxes represent additional domains. This collection of proteins is the result of BLASTP and PSI-BLAST searches (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) done using Aar, β-catenin, Arm and Vac8p protein sequences to identify similar proteins in *Arabidopsis*, combined with searches of the Pfam database (http://www.sanger.ac.uk/cgi-bin/Pfam/getacc?PF00514). All proteins have been cross-referenced with the MATDB database (http://mips.gsf.de/proj/thal/db/search/search_frame.html) to ascertain gene accession codes.



Box 1. *Arabidopsis* possesses proteins related to Aar, Arm and β-catenin

Arabidopsis proteins At2g44900 and At3g60350 (named Arabidillo-1 and Arabillo-2, respectively) show similarity to *Dictyostelium* Aar (PSI-BLAST first iteration score, 10^{-17}), β-catenin and Arm (10^{-10} to 10^{-9}). A representation of these proteins is shown in Figure Ia, where green boxes represent Arm repeats, yellow circles represent putative sites for GSK-3 phosphorylation ([S/T]-x-x-x-[S/T], where S is serine, T is threonine and x is any amino acid), and grey rectangles represent predicted F-boxes.

Below this representation (Fig. Ib) is an alignment of the three F-box sequences, done first by ClustalW [74] and followed by slight refinement by eye. The amino acid positions of the sequences in the full-length proteins are shown. The Pfam F-box consensus sequence is shown, and residues required for F-box function [63] are highlighted in grey. Residues identical to the consensus are shaded in red, conserved changes in blue. Residues that are conserved between Aar, Arabidillo-1 and Arabidillo-2 but are not the same as in the consensus sequence are shown in yellow.

Below the F-boxes (Fig. Ic) is an alignment of the nine Arm repeats of Arabidillo-1 and Arabidillo-2 and repeats 2–10 of *Dictyostelium* Aar done by ClustalW as above. The amino acid positions of the sequences in the full-length protein are shown. Each repeat is outlined in green, and the positions of the three α helices (H1, H2, H3) in each Arm repeat [2] are indicated by black lines above the alignment. Identical residues between proteins are shaded in red, conserved residues are shaded in blue.