Conserved residues in the ankyrin domain of VAPYRIN indicate potential protein-protein interaction surfaces

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Plants engage in mutualistic interactions such as root nodule symbiosis (RNS) with rhizobia and arbuscular mycorrhiza (AM) with Glomeromycotan fungi. These associations are referred to as endosymbioses because they involve transcellular passage through the epidermis and intracellular accommodation of the microbial partner within root cortical cells of the host. Infection by AM fungi and rhizobia is actively promoted by the plant and requires the establishment of infection structures namely the prepenetration apparatus (PPA) in AM and a preinfection thread in RNS, respectively. In both symbioses the intracellular microbial accommodation in epidermal and root cortical cells involves rebuilding of the cytoskeleton and of the entire membrane system. Recently, intracellular accommodation of rhizobia and AM fungi, and in particular morphogenesis of the AM fungal feeding structures, the arbuscules, was shown to depend on the novel VAPYRIN protein.

VAPYRINs are plant-specific proteins consisting of two protein-protein interaction domains, an N-terminal major sperm protein (MSP) domain and a C-terminal ankyrin (ANK) domain. MSP domains also occur in VAP proteins that are involved in membrane fusion processes in various eukaryotes. The ANK domain, on the other hand, closely resembles animal ankyrins which serve to connect integral membrane proteins to elements of the spectrin cytoskeleton, thereby facilitating the assembly of functional membrane microdomains in diverse animal cells. Ankyrin repeats exhibit features of nano-springs, opening the possibility that ankyrin domains may be involved in mechanosensing. Based on these structural similarities, VAPYRIN may promote intracellular accommodation of endosymbionts by interacting with membranes and/or with the cytoskeleton. Indeed, VAPYRIN protein associates with small subcellular compartments in petunia and in Medicago truncatula. Ankyrin repeats typically consist of 33 amino acids, of which 30–40% are...
the P. patens sequence fell out of the branch as in the case of repeats 4–6 (Fig. 2). Taken together, this points to an old evolutionary origin of the entire ankyrin domain in lower land plants, with no subsequent rearrangement of ankyrin repeats.

Ankyrin domains function as protein-protein interaction domains, in which the residues on the surface are involved in the binding of their protein partners. The fact that repeats 9 and 10 exhibited particularly high levels of conservation across species from moss to angiosperms indicated that this region may contain functionally important residues. Within repeat 10, sixteen amino acid positions were identical in >90% of the analyzed species (Fig. 3A and grey bars). Nine of those represent residues that are characteristic for ankyrin repeats (red letters) and determine their typical all the 12 species, it appeared that repeats 7, 9 and 10 exhibited particularly high conservation (Fig. 1C).

Sequence comparison of the eleven repeats of all the twelve plant species revealed that the individual repeats clustered according to their position in the domain, rather than according to their origin (plant species) (Fig. 2). This shows that the repeats each are well conserved across species, but show little similarity among each other within a given VAPYRIN protein. The higher conservation of repeats 9 and 10 was reflected by the compact appearance of the respective branches, in which the monocot and moss sequences were nested closely with the dicot sequences, compared to other repeats, where the branches appeared fragmented between monocots and dicots, and where the P. patens sequence fell out of the branch as in the case of repeats 4–6 (Fig. 2). Taken together, this points to an old evolutionary origin of the entire ankyrin domain in lower land plants, with no subsequent rearrangement of ankyrin repeats.

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A prominent clustering of VAPYRIN-specific residues was identified on the concave side of the crescent-shaped ankyrin domain comprising repeats 7–10 close to the gap (Figs. 3G and 4). This highly conserved VAPYRIN-specific region contains several negatively and positively charged residues (D, E and K, R, respectively) and aromatic residues (W, Y, F), which may together form a 3D shape. These residues are considered ankyrin-specific, and are unlikely to be involved in a VAPYRIN-specific function. The remaining seven highly conserved residues in repeat 10, however, are VAPYRIN-specific, since they have been under positive selection, without being essential for the basic structure of the ankyrin repeat. Ankyrin-specific and VAPYRIN-specific residues where identified throughout the entire ankyrin domain (Sup. Fig. 1), and subsequently mapped on a 3-dimensional model of petunia VAPYRIN to reveal their position in the protein (Fig. 3B–G). The ankyrin-specific residues were found to be localized primarily to the interior of the ankyrin domain, with the characteristic glycines (brown) marking the turns between helices and loops (Fig. 3B, D and F, compare with A). In contrast, the VAPYRIN-specific residues were localized primarily on the surface of the ankyrin domain (Fig. 3C, E and G). A prominent clustering of VAPYRIN-specific residues was identified on the concave side of the crescent-shaped ankyrin domain comprising repeats 7–10 close to the gap (Figs. 3G and 4). This highly conserved VAPYRIN-specific region contains several negatively and positively charged residues (D, E and K, R, respectively) and aromatic residues (W, Y, F), which may together form a
essential function of the C-terminal third
of the ankyrin domain, mutations that
abolish this relatively short portion of
VAPYRIN, have a strong phenotype, indi-
cating that they may represent null alleles.9
Based on this collective evidence, we
hypothesize that repeats 7–10 are involved
in the formation of a protein complex
that is essential for intracellular accom-
modation of rhizobia and AM fungi.
Biochemical and genetic studies are now
required to identify the binding partners
of VAPYRINs, and to elucidate their role
in plant endosymbioses.

Figure 3. 3D-Mapping of conserved positions within the ankyrin domain of VAPYRIN. (A) Conserved amino acid residues were evaluated for ankyrin repeat
10 of petunia VAPYRIN as an example. The degree of conservation between the 12 VAPYRINs analyzed in Figures 1B and 2 is depicted with grey bars. Average
conservation between all the 132 ankyrin repeats of the 12 VAPYRIN sequences is shown with black bars. Residues that are conserved in all 132 repeats
(red letters) define the ankyrin consensus sequence, which confers to the repeats their characteristic basic structure.17 Residues that are >90% conserved
but are not part of the basic ankyrin sequence (highlighted with asterisks) are VAPYRIN-specific and may therefore have been conserved because of their
specific function in VAPYRIN. Arrows indicate the characteristic antiparallel helices, the turns are marked by conserved glycine residues (underlined; com-
pared with B, D and F). (B–G) 3D-models of the petunia VAPYRIN PAM1. Conserved amino acid residues were color-coded according to their physico-
chemical properties (http://life.nthu.edu.tw/~fmhsu/rasframe/SHAPELY.HTM) with minor modification (see below). In (B, D and F) the ankyrin-specific residues are
highlighted (corresponding to the bold letters in Fig. 1A). In (C, E and G), the VAPYRIN-specific residues are highlighted. Note the patch of high conservation
on the concave side of the crescent-shaped ankyrin domain between repeats 7–10 next to the gap. (B–E) represent respective side views of the ankyrin
domain, (F and G) exhibit the concave inner side of the domain. Color code: Bright red: aspartic acid (D), glutamic acid (E); Yellow: cysteine (C); Blue: lysine
(K), arginine (R); Orange: serine (S), threonine (T); Dark blue: phenylalanine (F), tyrosine (Y); Brown: glycine (G); Green: leucine (L), valine (V), isoleucine (I), alanine
(A); Lilac: tryptophane (W); Purple: histidine (H); Pink: proline (P).

conserved binding site for an interacting
protein.
In this context, it is interesting to note
that human ankyrin R also contains a
binding surface on the concave side of
the D34 domain for the interaction with
the CBD3 protein.14 Consistent with an
essential function of the C-terminal third
of the ankyrin domain, mutations that
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