Targeting of Plant RanGAP to the Nuclear Envelope

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Introduction
The small GTPase Ran is one of the key players in nucleocytoplasmic transport. The directionality of this transport is determined by a gradient of RanGDP and RanGTP across the nuclear envelope. This gradient is formed by the spatial separation of RanGDP (Ran GTPase activating protein) outside and a nucleotide exchange factor inside the nucleus. In mammalian cells, the C-terminal domain of RanGAP is associated with the outer surface of the nuclear pore complex. This mammalian nuclear envelope-targeting domain is not present in yeast and plant RanGAP proteins, therefore suggesting a different mechanism or no targeting of RanGAP to the nuclear envelope in those organisms.

Plant RanGAP and MAF1 are targeted to the nuclear envelope in plant cells.

Identification of an interaction partner of the WPP domain.
The WPP domain-binding protein FLIP2 is a novel coiled-coil protein.

Figure 1: (A) Schematic comparison of the domain structure of vertebrate, yeast, and plant RanGAPs and MAF1 (HsRanGAP1, human; ScRanGAP1, S. cerevisiae; AtRanGAP1 and AAtMAF1, Arabidopsis). (B) Sequence alignment of Arabidopsis MAF and MAF-like sequences with the N-terminal domains of the two Arabidopsis RanGAPs. Blue shading indicates amino acid identity, yellow shading similarity. The highly conserved WPP motif is boxed in red.

Plant RanGAPs contain a unique N-terminal domain shared with the plant protein MAF1.

Figure 2: GFP (green fluorescent protein) fluorescence of tobacco BY-2 cells transiently expressing Arabidopsis RanGAP1-GFP (A) and tomato MAF1-GFP-MAF1 (B) fusion proteins. The MAF1 (MAF1-associated factor 1) sandwich construct was designed to prevent passive diffusion into the nucleus. See also figure on top next to title: Top left cell: AtRanGAP1-GFP, bottom right cell: LeMAF1-GFP-LeMAF1 SYTO 82 orange was used to counterstain for DNA (red), labeling the nucleus and nucleolus, as well as mitochondria and plastids in the cytoplasm.

A concentration of GFP fusion protein around the nuclear rim can be observed for both Arabidopsis RanGAP1 and tomato MAF1.

Figure 3: GFP fluorescence of BY-2 cells transiently expressing AtRanGAP1-GFP (A), AtRanGAP1-In-GFP (B), and AtRanGAP1-C-GFP (C).

Deletion of the WPP domain (B) leads to loss of fusion protein at the nuclear rim, whereas the WPP domain alone (C) is sufficient to target GFP to the nuclear envelope.

The WPP domain - a novel nuclear envelope targeting domain in plants.

The conserved WPP motif is required for nuclear-envelope targeting of Arabidopsis RanGAP1.

Figure 4: GFP fluorescence of BY-2 cells transiently expressing AtRanGAP1 mutants GFP (A), AtRanGAP1mut-GFP (B), and AtRanGAP1Cmut-GFP (C).

The mutation of the conserved WPP motif to AAP in AtRanGAP1 abolishes the targeting of the GFP fusion protein to the nuclear rim.

Conclusions and Outlook
We have found fundamental differences in plant and animal RanGAP targeting to the nuclear envelope, suggesting that different mechanisms have evolved for the spatial organization of Ran signaling in plants and animals. The consistency between nuclear-envelope targeting and binding of plant RanGAP to FLIP2 indicates that interaction with FLIP2 might be involved in anchoring plant RanGAP to the nuclear rim. This hypothesis and six additionally identified RanGAP-binding proteins from Arabidopsis are currently under investigation.

Literature