

A Novel Link between Ran Signal Transduction and Nuclear Envelope Proteins in Plants

Iris Meier*

Plant Biotechnology Center and Department of Plant Biology, Ohio State University, Columbus, Ohio 43210

A novel protein domain has been identified that is shared between putative plant Ran GTPase-activating protein (RanGAP) and a plant protein (MAF1) previously identified to be associated with the nuclear envelope. This domain is not present in RanGAPs from animals and yeast, suggesting that plant-specific protein-protein interactions might be involved in attaching RanGAP to the nuclear envelope.

The nuclear envelope separates chromatin from the cytoplasm and is involved in organizing nuclear architecture. It consists of two membranes (inner and outer) that are separated by the nuclear pore complexes (NPCs). Whereas the outer membrane is generally considered an extension of the endoplasmic reticulum, the inner membrane is characterized by a specific protein composition. A number of new inner nuclear envelope proteins have recently been discovered in animals. This group now includes lamin B receptor, lamina-associated polypeptide-1, lamina-associated polypeptide-2, emerlin, MAN1, otefin, and nurim (for review, see Wilson, 2000). In addition, the nuclear lamins, nuclear intermediate filament proteins, form a layer underneath the nuclear envelope and are connected to it by interactions with some of the integral membrane proteins such as lamin B receptor (Grant and Wilson, 1997). Several of these proteins have been shown to bind to chromatin, histones, and DNA, and they have been suggested to be involved in chromatin-nuclear envelope interaction during interphase. Based on their activities and localization, they are also candidates for proteins involved in nuclear envelope dynamics during open mitosis, such as the dissociation of the condensing chromatin from the nuclear envelope and the re-association of nuclear envelope vesicles around the decondensing chromatin (for review, see Grant and Wilson, 1997). The molecular mechanism of these processes is presently not known in any organism.

We have searched the higher plant sequences available in public databases, including the 88% of sequenced Arabidopsis genome, for potential homologs of the seven animal nuclear envelope proteins listed above as well as for lamin A/C and lamin B and have found no open reading frames with significant similarity to any one of them. This finding is consistent with the failure to successfully clone

plant lamins, although earlier reports using animal anti-lamin antibodies indicated that proteins with some similarity to lamins are present in plants (Beven et al., 1991; McNulty and Saunders, 1992; Minguez and Moreno Diaz de la Espina, 1993). Although the Arabidopsis genome is not completed and this analysis is therefore preliminary, it appears unlikely that all nine proteins are encoded on the remaining 12% of the genome. This might imply alternatively that plants have a different composition of proteins associated with their inner nuclear envelope. Multicellular plants and animals undergo open mitosis, whereas many unicellular eukaryotes like yeast go through mitosis with their nuclear envelope intact (Grant and Wilson, 1997). If a number of nuclear envelope proteins are involved in the orchestration of open mitosis, it would be conceivable that these proteins, like the process itself, have evolved twice in the animal and plant kingdom, thus explaining the presently observed lack of homologs of the animal proteins in plants.

Two plant proteins have been identified that are localized at the nuclear rim and are candidates for nuclear envelope-associated proteins. MFP1 binds matrix attachment region DNA and is a filament-like protein (Meier et al., 1996). However, unlike nuclear lamins, it does not have a typical intermediate filament protein structure, consisting of a central coiled-coil domain and globular head and tail domains. Rather, MFP1 consists of an extended coiled-coil domain that is preceded by an N terminus containing two hydrophobic, predicted transmembrane domains. The N terminus is necessary for the targeting of MFP1 to speckle-like locations at the nuclear rim, suggesting that MFP1 might be directly associated with the nuclear envelope membranes (Gindullis and Meier, 1999). MAF1 is a small novel Ser-Thr-rich protein that binds to the coiled-coil domain of MFP1. It is also located at the nuclear envelope, but in contrast to MFP1 it has a uniform distribution instead of a speckle-like pattern (Gindullis et al., 1999). We have proposed that MFP1 is involved in attaching chromatin through matrix attachment regions to the nuclear envelope (Gindullis and Meier, 1999). The potential function of MAF1 at the nuclear envelope is not known. Although both proteins are conserved among higher plants, they have no homologs in yeast or in animals, including the fully sequenced *Caenorhabditis elegans* genome. We had previously found

* E-mail meier.56@osu.edu; fax 614-292-5379.

no sequence similarity to other functionally characterized proteins from either plants or animals.

In a recent blast search with tomato MAF1 we have now uncovered a significant similarity of MAF1 to the N-terminal domain of recently identified putative plant RanGAPs. Ran is a small GTP-binding protein with an established function in yeast and animals in transport of proteins across the nuclear pore (Görlich and Kutay, 1999). RanGAP activates the GTPase activity of Ran and therefore the conversion of RanGTP to RanGDP. In animals, RanGAP is associated with the outer filament basket of the NPC through association with the NPC protein Nup358 (Yaseen and Blobel, 1999). In this location, RanGAP is involved in establishing the gradient of RanGDP to RanGTP between cytoplasm and nucleus that is required for nuclear import and export. Ran has been identified in plants (Ach and Gruissem, 1994; Merkle et al., 1994), but its function in nuclear import and export has not been elucidated (Smith and Raikhel, 1999). The first sequences for potential plant RanGAPs have been deposited recently (*Medicago sativa* RanGAP, AF215731; *Oryza sativa* RanGAP, AAD27557; *Arabidopsis* RanGAP1 [AtRanGAP1], AF214559; and *Arabidopsis* RanGAP2 [AtRanGAP2], AF214560).

All four putative RanGAPs contain an N-terminal domain with significant similarity to MAF1 from different plant species. This sequence similarity to MAF1 is missing in the RanGAPs from other organisms, such as *Drosophila melanogaster*, mammals, or yeast. Figure 1A illustrates as an example how the N terminus of AtRanGAP1 aligns with AtMAF1, whereas the rest of the protein aligns with hRanGAP, with the highest degree of similarity between the central domains of the two proteins. hRanGAP has no similarity to AtMAF1. Figure 1B shows an alignment between MAF1 sequences from six higher plant species and the N-terminal domains of the four RanGAP sequences. We have shown previously that the central part of MAF1 is most highly conserved, whereas the N terminus and C terminus are variable (Gindullis et al., 1999). We have found now that the domain that is conserved between MAF1 sequences from different species is also conserved among MAF1 and RanGAP sequences. A consensus sequence can be drawn from the alignment of the 10 sequences with 16 out of 90 residues being 100% conserved (diamonds in Fig. 1B). I suggest WPP domain as the name for the domain defined by this 16-amino-acid consensus sequence because of the highly conserved WPP motif. This domain establishes a clear relationship between the two kinds of proteins that is presently not shared by any other plant or animal sequences in the databases. Figure 1C shows the alignment of AtRanGAP1 with hRanGAP, illustrating why MAF1 and non-plant RanGAPs have no sequence similarity. In the N-terminal domain, the amino acids shared between AtRanGAP1 and hRanGAP are not identical to the amino acids conserved

between plant RanGAPs and MAF1 (diamonds mark the most highly conserved positions in the alignment shown in Fig. 1B).

What is the potential significance of this plant-specific N-terminal domain of RanGAP? And what is the functional connection between RanGAP and MAF1 in plants? The most straightforward answer to these questions appears to be that the WPP domain might be involved in protein-protein interaction. MAF1 interacts with the coiled-coil domain of MFP1, and MFP1 is associated with the nuclear envelope. Thus plant RanGAP might be associated with the nuclear envelope through interaction with MFP1. The localization pattern of MFP1 is reminiscent of nuclear pores (Gindullis and Meier, 1999). In animals, RanGAP is attached to the NPC through interaction with Nup358. At present, no homologs of Nup358 have been identified in plants. It is therefore possible that other interactions, like the binding of RanGAP to MFP1, are involved in plants to target RanGAP to the NPC.

Besides the well-established role of Ran in animal and yeast nucleocytoplasmic transport, several breakthroughs of the past months point toward a wider function of Ran in cellular signaling. Four groups have reported that RanGTP, but not RanGDP, can induce microtubule self-organization, and a novel Ran-binding protein has been found at the centrosome, indicating a role of Ran signaling in mitotic spindle organization (for review, see Kahana and Cleveland, 1999; Nishimoto, 1999). In addition, a second new role for Ran has now been described in nuclear envelope assembly (Hetzer et al., 2000; Zhang and Clarke, 2000). Using a combination of biochemical depletion and analysis of the effects of Ran mutants on *Xenopus laevis* egg nuclear envelope assembly, Hetzer et al. (2000) have shown that GTP hydrolysis by Ran is required for the early stages of nuclear envelope assembly. Zhang and Clarke (2000) have demonstrated that the coupling of RanGDP to sepharose beads in the absence of chromatin is sufficient to assemble a continuous double membrane containing functional NPCs in a cell-free *X. laevis* system. The authors propose that a high concentration of RanGDP at the end of mitosis will promote vesicle association with the decondensing chromatin.

Although our understanding of the role of Ran and its associated proteins in nuclear assembly is clearly at the earliest stage, it is tempting to suggest that RanGAP has to be specifically associated with the nuclear envelope vesicles at the end of mitosis. There it would locally provide the high rate of GTP hydrolysis and high concentration of RanGDP shown to be necessary for vesicle fusion. If the nuclear envelope-associated proteins involved in open mitosis do indeed differ fundamentally between animals and plants, then the association of RanGAP with the envelope vesicles might involve different interaction partners in the two kingdoms. In the light of the

this hypothesis by investigating the predicted protein-protein interactions and their temporal and spatial occurrence during cell cycle.

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LITERATURE CITED

- Ach RA, Gruissem W** (1994) *Proc Natl Acad Sci USA* **91**: 5863–5867
- Beven A, Guan Y, Pear J, Cooper C, Shaw P** (1991) *J Mol Biol* **228**: 41–57
- Gindullis F, Meier I** (1999) *Plant Cell* **11**: 1117–1128
- Gindullis F, Peffer NJ, Meier I** (1999) *Plant Cell* **11**: 1755–1767
- Görlich D, Kutay U** (1999) *Annu Rev Cell Dev Biol* **15**: 607–660
- Grant T, Wilson KL** (1997) *Annu Rev Cell Dev Biol* **13**: 669–695
- Hetzer M, Bilbao-Cortes D, Walther TC, Gruss OJ, Mattaj IW** (2000) *Mol Cell* **5**: 1013–1024
- Kahana JA, Cleveland DW** (1999) *J Cell Biol* **146**: 1205–1209
- McNulty AK, Saunders MJ** (1992) *J Cell Sci* **103**: 407–414
- Meier I, Phelan T, Gruissem W, Spiker S, Schneider D** (1996) *Plant Cell* **8**: 2105–2115
- Merkle T, Haizel T, Matsumoto T, Harter K, Dallmann G, Nagy F** (1994) *Plant J* **10**: 1177–1186
- Minguez A, Moreno Diaz de la Espina S** (1993) *J Cell Sci* **106**: 431–439
- Nishimoto T** (1999) *Biochem Biophys Res Commun* **262**: 571–574
- Smith HMS, Raikhel NV** (1999) *Plant Physiol* **119**: 1157–1163
- Wilson KL** (2000) *Trends Cell Biol* **10**: 125–129
- Yaseen NR, Blobel G** (1999) *J Biol Chem* **274**: 26493–26502
- Zhang C, Clarke PR** (2000) *Science* **288**: 1429–1432