Kinesin-Related Proteins with a Mitochondrial Targeting Signal[1]

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Conventional kinesin and kinesin-related proteins (KRPs) constitute a large family of microtubule-based molecular motors that play central roles in the transport of various vesicles and organelles in eukaryotic cells (Hirokawa, 1998). Mitochondrial movement also involves KRPs, which connect the mitochondrial surface with cytosolic microtubules (Nangaku et al., 1994; Pereira et al., 1997; Tanaka et al., 1998), although no KRP is known to target and work inside cytoplasmic organelles, including mitochondria. Here, we identify two similar KRPs from the higher plant Arabidopsis, named MKRP1 and MKRP2 (for mitochondria-targeted KRP), which contain an N-terminal mitochondrial targeting signal (MTS). They represent a new subclass of KRPs that might work within mitochondria.

In the Arabidopsis genome database, we identified two predicted genes, F8K7.17 and F19H22.150, that encode KRPs with an N-terminal extension. Both the extensions were predicted to function as an MTS by the computer algorithms MITOPROT (http://www.mips.biochem.mpg.de/cgi-bin/proj/medgen/mitofilter) and Predotar (version 0.5; http://www.inra.fr/Internet/Produits/Predotar/). We determined the full-length cDNA sequences using a PCR-aided strategy, and found that some spliced sites were inaccurately predicted. Both MKRP1 (corresponding to F8K7. 17; accession no. AB062738; 890 amino acids [aas]) and MKRP2 (corresponding to F19H22.150; AB062739; 1,055 aas) possess a conserved kinesin N-terminal motor domain and C-terminal coiled-coil domains, and are closely related to the KRP85/95 subfamily (Kim and Endow, 2000) based on the sequence similarity of the conserved motor domains (Fig. 1).

To determine whether the N-terminal extensions of MKRPs function as an MTS, expression vectors were constructed so that the N-terminal 162 aas of MKRP1 or 326 aas of MKRP2 were fused to the N-terminal end of green fluorescent protein (GFP; the soluble-modified red-shifted variant; Davis and Vierstra, 1998) and were highly expressed under the control of the cauliflower mosaic virus 35S promoter in plant cells. These vectors were introduced into tobacco (*Nicotiana tabacum*) leaf cells by the particle bombardment method. Transiently expressed chimeric GFPs as well as the *Saccharomyces cerevisiae* cytochrome oxidase subunit IV (coxIV) MTS:GFP, an efficient marker for visualizing plant mitochondria (Köhler et al., 1997), were localized in vesicular, sausage-shaped, or spaghetti-like bodies, depending on the cell types where GFP was expressed (Fig. 2, A–D). To confirm that these bodies were mitochondria, we co-introduced the MKRP1 N terminus:GFP and the Arabidopsis HSP60 (mitochondrial chaperonin) MTS (Prasad and Stewart, 1992) fused to the N-terminal end of CFP into tobacco. As a result, the GFP and CFP signals were superimposed completely (Fig. 2, E–P), suggesting that the N-terminal extensions of MKRPs have the ability to carry the entire proteins into mitochondria.

It is challenging to explore the function of MKRPs within mitochondria, which are believed to exclude microtubules. In yeast, mitochondrial molecules that regulate organelle morphology, fission, and fusion have been extensively described (Yaffe, 1999), but our BLAST analysis revealed that most of these yeast molecules do not have an Arabidopsis homologue (R. Itoh, unpublished data). MKRPs might play a role in plant-specific mitochondrial dynamics. It is suggestive that the *Escherichia coli* motor protein MukB has a domain structure similar to kinesin, and is involved in chromosome partitioning (Niki et al., 1991). By analogy, MKRPs might be involved in the segregation of mitochondrial nucleoids.

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LITERATURE CITED

Davis SJ, Vierstra RD (1998) Plant Mol Biol **36:** 521–528

Hirokawa N (1998) Science **279:** 519–526

Kim AJ, Endow SA (2000) J Cell Sci **113:** 3681–3682

Köhler RH, Zipfel WR, Webb WW, Hanson MR (1997) Plant J **11:** 613–621

Nangaku M, Sato-Yoshitake R, Okada Y, Noda Y, Takemura R, Yamazaki H, Hirokawa N (1994) Cell **79:** 1209–1220

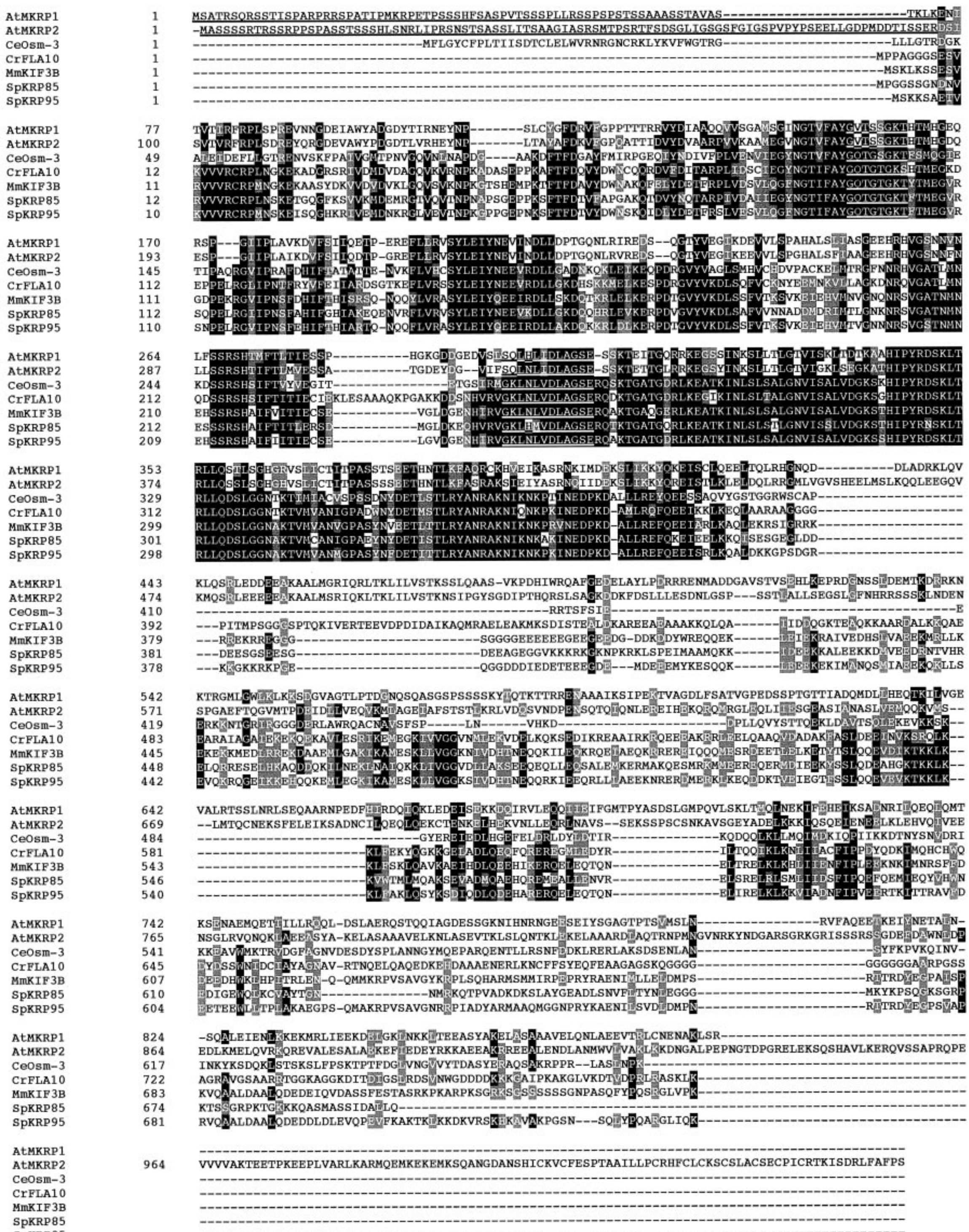

Figure 1. Alignment of the KRP85/95 subfamily proteins from several organisms. The alignment was performed with ClustalW 1.8 using the default parameters shown at the web site http://searchlauncher.bcm.tmc.edu/multi-align/Options/clustalw.html. The N-terminal extensions characteristic of Arabidopsis MKRPs are underlined. A conserved ATP/GTP-binding site motif A (P loop; G-[V/Q]-T-[S/G]-[S/T]-G-K-[T/S]) and a conserved kinesin motor domain signature ([S/G]-[Q/K]-L-[H/N]-[L/M]-[I/V]-D-L-A-G-S-E) are indicated by highlighted underlines. At, Arabidopsis; Ce, *Caenorhabditis elegans*; Cr, *Chlamydomonas reinhardtii*; Mm, *Mus musculus*; Sp, sea urchin (*Strongylocentrotus purpuratus*).

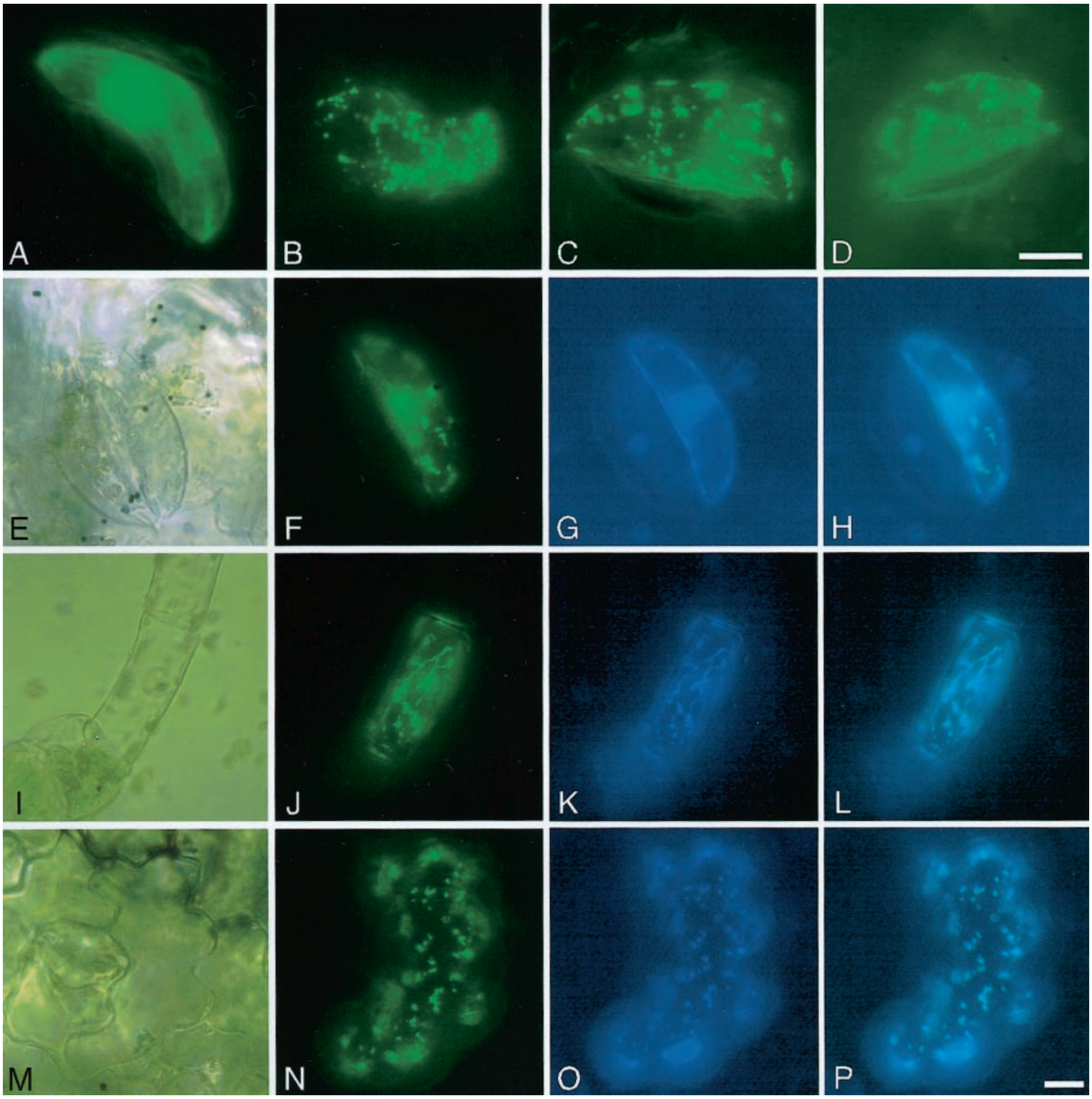

Figure 2. Mitochondrial localization of GFP fused with the N-terminal regions of MKRPs. The fusion proteins were expressed in tobacco leaf cells. A through D, Fluorescence images of various GFPs expressed in guard cells, taken at the same magnification. A, Non-fused GFP as a negative control, dispersed over the entire nucleo/cytoplasm. B, The N-terminal 29 aas of coxIV fused to GFP, as a positive control. C and D, MKRP1 (C) and MKRP2 (D) N terminus:GFP, both confined in small vesicular organelles as observed in B. E through P, Co-expression of MKRP1 N terminus:GFP and cyan fluorescent proteins (CFPs), taken at the same magnification. E through H, The chimeric GFP and non-fused CFP co-expressed in a guard cell as a negative control. Images of bright field (E), GFP (F), CFP (G), and the GFP/CFP overlay (H) are shown. CFP signals are observed in the nucleoplasm and at the periphery of the cytoplasm. Note that vesicular GFP signals are substantially excluded through the cyan channel, indicating the separation of green and cyan fluorescence. I through P, The chimeric GFP and N-terminal 60 aas of HSP60 fused to CFP, co-expressed in trichome (I–L) and epidermal (M–P) cells. Images of bright field (I and M), GFP (J and N), CFP (K and O), and the overlay (L and P) are shown. Colocalization of GFP and CFP is visualized in sausage-shaped (L) and spherical (P) organelles. Bar = 10 μm.

Niki H, Jaffe A, Imamura R, Ogura T, Hiraga S (1991) EMBO J **10:** 183–193

Pereira AJ, Dalby B, Stewart RJ, Doxsey SJ, Goldstein LSB (1997) J Cell Biol **136:** 1081–1090

Prasad TK, Stewart CR (1992) Plant Mol Biol **18:** 873–885

Tanaka Y, Kanai Y, Okada Y, Nonaka S, Takeda S, Harada A, Hirokawa N (1998) Cell **93:** 1147–1158

Yaffe MP (1999) Science **283:** 1493–1497