Kinesin-Related Proteins with a Mitochondrial Targeting Signal¹

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Conventional kinesin and kinesin-related proteins (KRPs) constitute a large family of microtubulebased 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

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end of green fluorescent protein (GFP; the solublemodified 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, sausageshaped, 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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at www.m.m.1		
AtMKRP1	1	MSATKSQRSSTISPARPRKSPATIPMKKPETPSSSHF5ASPVTSSSPILKSSPSPSISSAAASSIAVAS
AtMKRP2	1	-MASSSSRTRSSRPPSPASSTSSSHLSNRLTPRSNSTSASSLITSAAGIASRSMTPSRTFSDSGLIGSGSFGIGSPVPIPSEELLGDPMDDTISSER
CeOsm-3	1	KINTERSTATESMFLGYCFPLTIISDTCLELWVRNRGNCRKLYKVFWGTRGCALLGTRUGK
CrFLA10	1	MPPAGGGS
MmWTE3D	1	MSKLKSS PSV
MIRTESB	1	UDCCCCCX WAY
SpKRP85	1	MPGGSSGNØN
SpKRP95	1	MSKKSABav
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ATMKRPI	//	
AtMKRP2	100	SVITVRFRPISDRBYQRGDEVAWYPIGDTLVRHEYNPLTAMAPDKVEGPEATTIDVYDVAARPVVKAAAHGVNGTVFATGVISSGKTHTUHODQ
CeOsm-3	49	ATENDEFLIGTRENVSKFPAHVGHTPNVGQVNINABDGAAKDETHDGAMFMIRPGEQHVNDIVFDIVENVAHGYNGTVFAYGONHSGKTESMOGIE
CTFLA10	12	KVVVPC PPI NGKEKADGRSRIVDADVDAGOWKWRNPKADASEPPKAFTFDOVYDWICOORDVEDITARPLHESCIEGYNGTI FAYGOTGTGKSHTMEGKD
W-WIR2D	11	THE REPORT OF TH
MILKIFSB	11	AVVVACAPHINGAMAAAS I DAVID VI VALISA VI V
SPKRP85	12	RVVVRCRPLNSKETGOGFKSVVKNDBARGIVOVINPNEPSGEPPKSFTFDIVTAPGAROTDVYRCTARPTVBACTEGAROTTPATGOTOGATETMBGAR
SpKRP95	10	KVVVRCRPMNSKEISQSHKRIVEUINKRELVEVINEKCPPGBPNKSFTFDTVYDWNSKQIDEYDEIFRSIVESWEGENGTIFAYGOTGTGKTETHEGWR
A+WYDD1	170	PORTAGE AVAILABLE AVAILABLE AVAILABLE AVAILABLE DE CONTRACTOR
ACHKRPI	170	
AtMKRP2	193	ESPGHTELAIKDVFSIIIODHP-GREPLIRVSYLETYNEVINDLIDPIGONLRVREDSOGHYVEGIKEEVVLSPGHALSPHAAGEERNHVGSNNFN
CeOsm-3	145	TI PAQ RGVI PRAFIDIII FYATATTE – NVKFLVHCSYLEI YNEEVRDILLGAD XKQKLEI KEODIRGVYVAGHSMHVCHDVPACKEHATRGPNNRHVGATDAN
CrFLA10	112	EPPELRG& I PNUTRYVET I ARDSGTKEFLVRSSYLE I YNEEWRDLLGKDHSKKMELKESPDRGVYVKDLSQFVCKNYEBMNKVULAGKDNRQVGATLMN
MmKTF3D	111	CODEXEGVIENSEDHIEFHISESO, NOOVIVEASVIEIVÕEEIRDLISKOOTKELEIKERPDIGVVVKDLSSEVIKSVKEIEHVMINVGNONRSVGATNMN
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SpKRP85	112	SOPELRGTIPNSFAHTFGHTAKEOENVRFLVRVSYLETYNEEWROLLIGKDOERLEVKDRPUVGVYVKDLSAFVVNNADDSDRTHTTGHNARASVOATNAN
SpKRP95	110	SNPELRGVIPNSFEHIF#HIARTQ-NQQFLVRASYLEIYQEEIRDLLAKDQKKRLDBKERPDTGVYVKDLSSFVTKSVKBHEHVMTVGNNNRSVGSTNNN
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A+MKPD1	264	LESSESHMERTIN ISSPHGKGDGEDUSISOFHITIDIAGSE-SSETET TOORREGISTIKKUT TIGOTYISKITTOTKAALI PVRDSKTT
ACHART I	204	
AtMKRP2	287	LLSSRSHRTHTTM/HSSATGDEYBGVIFSONTHTDMARSH-SSRAFTHGLRRAMSHTHTLGINGSENAVHTPTRDSRLT
CeOsm-3	244	KDSSRSHSTFTVYVDGTTBTGSTRM <u>GKINLVDLAGSE</u> ROSKTGATGDRLKEATKINLSLSALGNVISALVDGKSKHIPYRDSKLT
CrFLA10	212	ODSSRSHSIFTITIECTEKLESAAAOKPGAKKDDSNHVRVGKLNLVDLAGSERODKTGATGDRLKEGIKINLSLTALGNVISALVDGKSGHIPYRDSKLT
MmVTF2D	210	VGLOCENHER OF VGLOCENHERVGKLNLVDLAGSEROAKTGAOGERLKEATKINLSLSALGNVISALVDGKSTHIPYRDSKLT
MILKIP 3B	210	
SpKRP85	212	ESSSRSHATFTTTTERSEMGDDKEOHVRVGKDHWVDLAGSEROTKTGATGORLKEATKINDSLSTCGNVISSLVJGKSTHTPTRUSKDI
SpKRP95	209	EHSSRSHAIFIITIECSELGVDGENHIRV <u>GKLNLVDLAGSE</u> RQAKTGATGDRLKEATKINLSLSALGNVISALVDGKSSHIPYRDSKLT
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AUMKRPI	353	
AtMKRP2	374	RIALOSSI SCHEHIVSIMICTI TIPASSSSIDETHINI KEASKAKSTETYASRNOTTIDIKSHI HAKMORDISTI.KLEHIDQLKROMLVGVSHEELMSLKQQLEEGQV
CeOsm-3	329	RLLODSLGGNTKTIMIACWSPSSCNYDETLSTLRYANRAKNIKNKPTINEDPKDALLLREYQEESSAQVYGSTGGRWSCAP
CrFLA10	312	RLLODSLGGNTKTVMVANIGPADWNYDETMSTLRYANRAKNIONKPKINEDPKD-AMLRØFOEEIKKLKEOLAARAAGGGG
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MMK1F3B	299	RELIGISE LIGRARY TY MY ANY GPAS IN MEETERTER TANKAKINI KINK PROMEDPAL - ALLINE FORE TANKAGULARS I GARA
SpKRP85	301	RLLODSLGGNAKTVMCANTGPAEVANDETTISTIERVANRAKNTKNKAKTNEDPKD-ALLEREFOKETEEDEKKOUSESGBGLDD
SpKRP95	298	RLLODSLGGNAKTVMVANMGPASYNEDETTTTLRYANRAKNIKNKPKINEDPKC-ALLREFQEEISRLKQALDKKGPSDGR
		THE REAL PROPERTY AND
AtMKRP1	443	KLQSRLEDDM#9KAALMGRIQRLTKLILVSTKSSLQAAS-VKPDHIWRQAF4E8ELATLP%RKRENADDGAVSTVSSHLADAS39DEHTRD/RKK
AtMKRP2	474	KMQS@LEBEBEEKAALMSRIQKLTKLILVSTKNSIPGYSGDIPTHQRSLSAKK@DKFDSLLLESDNLGSPSST@ALLSEGSL@FNHRRSSS&LNDEN
CeOsm-3	410	RRTSFSI
CrFLA10	392	
CIFERIO	372	
MMK1F3B	379	
SpKRP85	381	DEESGSBESGDEEAGEGGVKKKRKEKNPKRKLSPEIMAAMQKK
SpKRP95	378	KIGKKRKPEE
1.1.1.1.1.1	540	
AtMKRP1	542	KTRGALLWINGLAGSDIGVAGTLPTDENQSQASGSPSSSSKI QTKTTRAGAAAAIKSIPEGIVAGDLFSATVGFEDSSFIGTTRADABA
AtMKRP2	571	SPGAEFTQGVMTPD2IDintVEQVKMiAGEnAFSTSTiKRLVD%SVNDP2%SQTQmQNLE3EIHEKQ2Q%RGLmQL1im2SGBAS1@NAS1@NAS1@NAS1@NAS1@NAS1@NAS1@NAS1@N
CeOsm-3	419	RKINTCRIEGGGDERLAWRQACNASSFSPLNVHKD
CTELA10	483	BARATAGARGERERE CORACINE STREETER CONTRACTOR AND A CONTRACT
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MMK1F3B	445	REFARED STREND ARE IN GAN WARDAN WORKN VID. AND OKTING AND A THE REFARED AND A THE AND A
SpKRP85	448	BLORRESEMIKAQDDQKIIINEKENAIQKKAIIVCCVDIIIAKSEDQEQLLIBOSALEMKERMAKQESMRKMAIBERIQERAIILEEKMSSTQDBAHGKIKKLA
SpKRP95	442	EVORROSENTKETQQKENLEGK KAMESKIPVGGKSIVDHINEQQRKIEQQLLIAEEKNRER MERKIKEQIDKTWIEGTESSLQQEVEWKTKKLK
A4WEDD1	642	
ACMARTI	042	TALKISSINGLESSYARKEEDE THE DURANTE DER AND THE TABLE TO THE RESIDENCE TO THE TABLE
AtMKRP2	669	LMTQCNEKSFELEIKSADNCIIQEQUQEKCTENKERHEKVNLLEQUURAASSEKSSPSCSNKAVSGEIADELKKKUQSQEHENBELKLEHVQIVEE
CeOsm-3	484	KQQQWKLLMQUWKIQBIIKKDTNYSN@DRI
CTFLA10	581	
MmWTF2D	542	KLESKI OAVKARTHDI OMEH LERRORI ROTON
FINELT SB	543	
SPKRP85	546	KVII/TEIMOAKSENARIAUKIARHUSINEEARIISNYKEISKEIKKISMIITEISSUUOBFOEMILOI VHIM
SpKRP95	540	EUIRELKIKKVIADNELEVENTIODIODELARDELEOTQNEUIRELKIKKVIADNELEVERTKUTTRAVED
A+MKPD1	742	KSEINAEMORTHITLLREOL-DSLAFROSTOOTAGDESSGKNTHNRNGREBSETYSGAGTPTSEMSLE
A LANDON	746	NOCT DVO NOT MED OVA VET ACAAVET VAT ACEVITYL CLONTER HEFT AAADD ACTIVIDUNGUND VADCAD COD COT COC CO COT CONTRACTOR
ATMKRP2	765	NSGLKVUNQUIREETIS IN-RELASAAAVELKNLASEVIKLSLQUIKLIMKELMAARDIAQIKKPIKGVIKKINLSAKSGKGKISSSKSGGDEHDUWNDD
CeOsm-3	541	KKEAVMMKTREDGFEGNVDESDYSPLANNGYMQEPARQENTLLRSNFEDKLRERLAKSDSENLAN
CrFLA10	645	EYESSENEDCENYAEXAV-RTNQELQAQEDKEEDAAAENERLKNCFFSYEQFEAAGAGSKQGGGGGGGGGGAERPGSS
MmKTF3D	607	REDHOKEHPETRIEN_O_OMMKRPVSAVGYKEPISOHARMSMMIRPEPPRAENTELLERDMPSRETTEDTREPARS
A-WDDOG	507	
SpKRP85	610	DIGENORCYNYTENRAINFOCTPVADKDKSLAYGEADLSNVFWTIN EGGGRAINFOCSASGA
SpKRP95	604	≝ETEEML#TP#MKAEGPS-QMAKRPVSAVGNR©PIADYARMAAQMGGNPRYKAENI™SVD∰DMPNR#TRD₩E©PS₩AE
A+MEDD1	024	SOM R TENN SKEKNEL TEEKDERGKENKKETERASYAKELON AREUTELON AREUTELON RAMISS
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AtMKRP2	864	EDLKMELQVREVALESALASKEFTEDESIKKAREEARIKEDEBLENDLANMWVIVAKUSKUNGALPEPINGTDPGKELEKSQSHAVLKERQVSSAPRQPE
CeOsm-3	617	INKYKSDQKESTSKSLFPSKTPTFDG@VNG@VYTDASYE@AQS&KRPPRLAS@N@&
CrFLA10	722	AGRAVGSAARETGGKAGGKDITDIGSERDSENWGDDDDKEKEATPKAKGLVKDTEDERLEASKLK
MmKTE3D	693	KYONALDAADORDETOYDASSEESTASEKPKARPKSGRISESSSSGNDASOFYDOSEGT.VD3
FINAL F 3B	005	MANUNDAMANANA AN ALTA AL
SpKRP85	674	KTSSGRPKTGRKKQASMASSIDATLQ
SpKRP95	681	RVQMALDAAMQDEDDLDLEVQP@WFKAKTK@KKDKVRSK@K@V%KPGSNSQ@YPQA&GLIQK
2		
A+MVDD1		
ACMARPI		THE ALL AND A REAL AND A
AtMKRPZ	964	vvvvartestfresplvarlkarmqemkekemksqangdanshickvcfesptaallepckhfchckscslacsscficktrisDklfafps
CeOsm-3		
CrFLA10		
MmKTF3B		
CoVDDOF		
SPKRP85		
SpKRP95		

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 μ m.

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