A novel isoform of sarcolemmal membrane-associated protein (SLMAP) is a component of the microtubule organizing centre

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Summary

The microtubule organizing centre (MTOC) or the centrosome serves a crucial role in the establishment of cellular polarity, organization of interphase microtubules and the formation of the bipolar mitotic spindle. We have elucidated the genomic structure of a gene encoding the sarcolemmal membrane-associated protein (SLMAP), which encodes a 91 kDa polypeptide with a previously uncharacterized N-terminal sequence encompassing a forkhead-associated (FHA) domain that resides at the centrosome. Anti-peptide antibodies directed against SLMAP N-terminal sequences showed colocalization with γ-tubulin at the centrosomes at all phases of the cell cycle. Agents that specifically disrupt microtubules did not affect SLMAP association with centrosomes. Furthermore, SLMAP sequences directed a reporter green fluorescent protein (GFP) to the centrosome, and deletions of the newly identified N-terminal sequence from SLMAP prevented the centrosomal targeting. Deletion-mutant analysis concluded that overall, structural determinants in SLMAP were responsible for centrosomal targeting. Elevated levels of centrosomal SLMAP were found to be lethal, whereas mutants that lacked centrosomal targeting inhibited cell growth accompanied by an accumulation of cells at the G2/M phase of the cell cycle.

Key words: Sarcolemmal membrane-associated protein (SLMAP), Centrosome, Forkhead associated domain, Genomic structure, Cell cycle

Introduction

The microtubule organizing centre (MTOC) of the cell is the centrosome, a complex organelle that fulfills multiple functions including the nucleation of interphase microtubules, the establishment of cellular polarity, the formation and positioning of the bipolar mitotic spindle, and the segregation of chromosomes (reviewed by Doxsey, 2001; Nigg, 2002). Failure to preserve the integrity of centrosome structure and activity has severe consequences on cell cycle progression and genomic stability (Whitehead and Salisbury, 1999; Kaiser et al., 2002; Doxsey, 2001). Deregulated centrosome activity has been shown to promote the missegregation of chromosomes, which may lead to aneuploidy, a common feature of tumors (Doxsey, 2002; Nigg, 2002). In view of the pivotal role of the MTOC in ensuring genomic stability, various kinases and phosphatases anchored at centrosomes function as central regulators of centrosome activity (Meraldi et al., 1999; Hinchcliffe et al., 1999; Sluder and Hinchcliffe, 2000; Nigg, 2002). Examples include p34^{cdc2}, cAMP-dependent kinase II (PKA), Polo kinase, Cdc14A phosphatase, STK15(BTAK) and Nek2 kinase (reviewed by Mayor et al., 1999; Meraldi and Nigg, 2002; Mailand et al., 2002; Whitehead and Salisbury, 1999). While the functional roles of many centrosomal kinases and phosphatases continue to be further defined, the repertoire of centrosome-associated substrates remains to be uncovered.

In recent years, considerable progress has been made in elucidating the protein composition of centrosomes. In this regard, proteins localized to the MTOC have been classified as: (1) integral components of centrosomes; (2) proteins associated with centrosomes in a transient, cell cycle regulated manner; and/or (3) proteins that require microtubules for centrosomal associations (Urbani and Stearns, 1999). Coiled-coil structure has emerged as a common structural motif predicted in several proteins localized at the MTOC, among which include pericentrin, Nek2 protein kinase, ninein, CG-NAP, kendrin and p160-Rho-associated coiled-coil-containing protein kinase (ROCK) (Doxsey et al., 1994; Fry et al., 1998; Fry et al., 1999; Boukson-Castaing et al., 1996; Takahashi et al., 1999; Takahashi et al., 2002; Li et al., 2000; Chevrier et al., 2002). Further characterization of novel structural and regulatory components localized at the MTOC will foster an improved understanding of the complex mechanisms defining centrosomal functions.

Sarcolemmal membrane-associated proteins (SLMAPs) comprise a unique family of alpha-helical coiled-coil proteins encoded by a single gene mapped to human chromosome 3p14.3-21.2 (Wigle et al., 1997; Wielowieyski et al., 2000). Elucidation of the genomic organization of the 3′ region of the SLMAP gene indicated the presence of several splice variants, which exhibit developmental and tissue specific expression (Wielowieyski et al., 2000). A central coiled-coil region encompassing two leucine zipper motifs constitutes the core structural feature of all SLMAP isoforms, together with a single transmembrane domain at the C-terminus that can be alternatively spliced to target these polypeptides to cellular membranes (Wielowieyski et al., 2000). In the present study

Table 1. Exonic primer sequences used to clone SLMAP genomic fragments

Primer	Sequence
*708-F (22mer)	TTA CCC ATG GGT GTA TTG TTT C
*822-R (28mer)	GCA ACT TTG TCT ACA GGA CTC GGT AAT G
*781-F (24mer)	AGA TGT CAT CCA TGC TCC ATT ACC
*829-F (23mer)	CAC TCC AAG TAT GTA CTC TCA GG
*851-R (23mer)	CCT GAG AGT ACA TAC TTG GAG TG
*922-F (22mer)	CCA CAC TTC AGC GGC TAC TAG C
*966-R (24mer)	GTA TCT GAA CCC TCT TGG GTG ATG
†1023-R (24mer)	CCC ATA ACT TCT AAC CGT GAT AAG
†1062-R (29mer)	TCT TCT GTT TGA TTT TTG GAG CAT GCC TG
†1140-R (22mer)	CGC CTC AGG GAC TCT TTG GCT G
†1228-R (23mer)	CAG GTG GGT ACA TTC ATC TTC AG
*1377-R (24mer)	TTC TCA GCT TGT CCC TTC TGT TGG
*Ex1-F (36mer)	AGA AGC TGA TCG TGG AGG GGC ATC TAA CCA AAG TGG
†Ex2-F (21mer)	AAA ATC AGG CAA AAG CAA AGG
†Ex2-R (18mer)	CTG TAG TGT CGT CGC TGC
*Ex3-R (38mer)	CTT TTA ATA GAG ACA CCT TAG CAA GGG GTT CAT TTA GG
†Ex4-F (32mer)	ACT TAC AGG GTA CCC AGT CAG AAA CTG AGG CC
†Ex4-R (33mer)	CTT GAA GTT CAA AGC ATT TTT GTT TAC TTG TTC
†Ex5-F (19mer)	CTC TTT TGG AAG AAG AAA G
†Ex5-R (19mer)	CTT GAA GAA CCT GTA TTT G
†Ex6-R (33mer)	ACC TTG TTG CCT CCT CCC TTT GCT GGT CTT CAC
†Ex7-F (20mer)	GTG AGC TGG AGA AGT TGA AG
†Ex7-R (22mer)	TTG TGC AAT TCT TTC TCT TGT C
‡Ex11-R (25mer)	GGT GCA CTA AGA AGC GTA CTT TAG G
‡1-15F (25mer)	CTC CAG CCC AAA ACT GCT TCC AGA G
‡405-F (27mer)	ACC CGT TTC AGG AGC GTC ATG TCT ACC

*Used for PCR cloning.
†Used for internal verification of genomic library clones and/or large PCR clones.
‡Used to generate SLMAP cDNA probe.

we elucidated the genomic organization of the entire SLMAP gene, which was found to comprise 24 exons spread over ~122 kb of DNA. The genomic structure of the SLMAP gene predicted the presence of several splice variants, one of which was found to be a component of the MTOC.

Materials and Methods

Isolation and sequence analysis of genomic clones.

Genomic clones corresponding to the SLMAP gene were isolated by direct screening of two mouse genomic libraries (λFIXII and λDASH II; Stratagene). SLMAP cDNA probes used for library screening were PCR generated (Table 1) and digoxigenin labeled, according to the manufacturer's directions (Roche Applied Science) for subsequent use in Southern blotting experiments. Positive phage DNA clones were digested and resolved by electrophoresis. Restriction enzyme digest fragments of the phage genomic DNA clones were analyzed by Southern blotting to identify SLMAP exons. Genomic DNA for each of the eight clones was isolated (Table 2), subcloned into pBlueScript KS (Stratagene) and subsequently sequenced using AmpliTaw sequencing methodology (ABI). Sequencing of the identified exons enabled the identification of exon-intron junctions by PCR using forward and reverse primers designed to span successive exons (Table 1). PCR conditions consisted of the following steps: (1) 3 minutes at 94°C; (2) 30 cycles of denaturation at 94°C for 30 seconds, annealing at 50-55°C for 30 seconds, extension at 72°C for 1-3 minutes; and (3) 10 minutes at 72°C. Amplicons were resolved by electrophoresis, and the isolated DNA was cloned into the pCR4-TOPO cloning vector (Invitrogen) for subsequent sequence analysis. Computer-assisted alignment of SLMAP cDNA sequence (Accession #AF304451) with genomic sequences facilitated the identification of putative exons. The exon-intron organization of SLMAP3 was verified by aligning the mouse cDNA sequence of SLMAP3 (Accession AF304451) with mouse genomic sequences (NW 000090.1) deposited in GenBank. Consensus phosphorylation, N-glycosylation and N-myristoylation sites were identified using PROSITE database available on the EMBL server (Falquet et al., 2002).

Cell culture, transfections and drug treatments

NIH 3T3 fibroblast cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 50 units/ml penicillin, 50 μg/ml streptomycin and gentamycin at 37°C in a humidified 5% CO_2 atmosphere. Transient transfection experiments were performed using the LIPOFECTAMINE PLUS™ (Gibco BRL) transfection reagent according to the manufacturers' specifications. For experiments where microtubules were disrupted, NIH 3T3 cells grown on sterile glass coverslips were treated with either nocodazole (Sigma Chemical Co., 6 μg/ml) or paclitaxel (Sigma Chemical Co., 4 μM) in growth media (DMEM) for 3 hours at 37°C.

Antibodies

Two polyclonal antibodies against SLMAP were generated by injecting rabbits with two synthetic SLMAP-specific peptides. Anti-SLMAP(C) rabbit antisera was raised against the carboxyl 370 amino acids of SLAP, as previously described (Wigle et al., 1997). A second antibody, designated anti-SLMAP(N), was raised against the peptide RLSRGEESPPCEI, which corresponds to the extreme N-terminus of SLMAP3, within the region separating two different initiating methionines M1 and M2 (Fig. 2A). Monoclonal anti-γ-tubulin (clone GTU-88, Sigma Chemical Co.) was used to identify centrosomes, and monoclonal anti-α-tubulin (clone DM 1A, Sigma Chemical Co.) was used to visualize cytoplasmic microtubules in immunocytochemical studies. The DNA stain 4′,6-diamidino-2-phenylindole dihydrochloride (DAP1) was purchased from Molecular Probes. Secondary antibodies used in immuno-cytochemistry studies included FITC-conjugated anti-rabbit immunoglobulins (Amersham

Table 2. List of genomic clones containing SLMAP exons and the methods of isolation

Clone (size)	Exons encompassed	Method obtained
A (10 kb)	Exon II	Direct screening of genomic library*
B (3.6 kb)	Exon IV to Exon V	PCR cloning (708-F & 822-R)†
C (2.0 kb)	Exon V to Exon VI	PCR cloning (781-F & 851-R)†
D (3.0 kb)	Exon VI to Exon II	PCR cloning (829-F & 966-R)†
E (6.0 kb)	Exon VII to Exon IX	PCR cloning (922-F & 1377-R)†
F (4.0 kb)	Exon XIV to Exon XVI	PCR cloning (Ex1-F & Ex3-R)†
G (20 kb)	Exon XV to Exon XXI	Direct screening of genomic library‡
H (4.5kb)	Exon XIII to Exon XV	Direct screening of genomic library‡

*λ FIX II mouse genomic library (Stratagene).
†The exon-intron junctions of SLMAP3 and sizes of the respective exons and introns.
‡λ DASH II mouse genomic library (Stratagene); see Table 3.

Pharmacia Biotech) and CY3-conjugated anti-mouse immunoglobulins (Jackson ImmunoResearch Laboratories). Secondary antibodies used in immunoblotting experiments included anti-rabbit IgG peroxidase linked whole antibody (Amersham Pharmacia Biotech) and peroxidase conjugated AffiniPure goat anti-mouse IgG (Jackson ImmunoResearch Laboratories).

Immunoblot analysis

NIH 3T3 cells were solubilized by RIPA lysis buffer (1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS in PBS pH 7.4) and centrifuged at 10,000 ***g*** (15 minutes, 4°C). The protein content of clarified supernatants was determined using the BCA Protein Assay Kit (Pierce). Proteins (15 μg) were resolved by electrophoresis on 10% SDS-polyacrylamide gels, according to Laemmli (Laemmli, 1970). Separated proteins were electrophoretically transferred onto PVDF membranes (Amersham Pharmacia Biotech) and blocked in 5% skim milk powder (SMP) in TRIS-buffered saline containing 0.05% Tween-20 (TBS-T), then incubated at room temperature for 1 hour with anti-SLMAP rabbit antibodies (1:4500) in 5% SMP/TBS-T. After four washes in TBS-T, blots were incubated with anti-rabbit IgG peroxidase linked whole antibody (Amersham Pharmacia Biotech) in 5% SMP in TBS-T for 1 hour. Antibody detection was carried out using the enhanced chemiluminescent detection system (Amersham Pharmacia Biotech).

Isolation of centrosomes

Centrosomes were isolated from NIH 3T3 cells using sucrose density gradients according to the method of Moudjou and Bornens (Moudjou and Bornens, 1998).

Mammalian expression plasmids

SLMAP3M1 (nucleotides 1-2314) was PCR generated from the original full-length SLMAP rabbit cDNA clone (Wigle et al., 1997) using the forward primer SLMAPN-F (GGAATTCGATG-CCGTCAGCCTTGGC) and reverse primer SLMAPN-R (GATG-CCAGCTTCTAGAGGGAGGACG). Forward primer SLMAPN-F and reverse primer (SLMAP+3′) CCTCTAGAGCTCAGCTCTCAC-CTTCTTAAGC were utilized to produce carboxyl truncation mutant (nucleotides 1-1674), designated SLMAP3M1ΔC. The generation of N-terminal/C-terminal truncation mutant SLMAP3M2ΔC (nucleotides 389-1674) was accomplished using forward primer (ATG2M5′) GGAATTCAGATGGTATGGAAGCC and the reverse primer (SLMAP+3′). The PCR products were each inserted into the *Eco*R1 and *Xba*1 sites of vector pcDNA3 (Invitrogen), in frame with the open reading frame of the green fluorescent protein (GFP). Sites of ligation were confirmed by DNA sequencing. To generate the leucine zipper (LZ) mutant (GFP-SLMAP3M1ΔLZ), the GFP-SLMAP3M1 construct was digested with *Bam*H1 to release the segment of SLMAP that included the leucine zippers (nucleotides 1-1994). We retained the vector that included nucleotides 2001-2314 of SLMAP3M1 (designated SLMAP3′) for subsequent subcloning of a PCR amplicon of SLMAP3M1 lacking the leucine zippers. This amplicon was generated using GFP SLMAP3M1 template and employing primers GFP-5′ (GGGATCCATGGACAAAGGAGAA-GAACTCTTCAC) and antisense primer LZ-less 3′ (CGGATC-CCTCTTTCTGCTGGTCCTCACACTGC), which both incorporated *Bam*H1 sites. The PCR product (lacking the leucine zippers) was restriction digested (*Bam*H1) and then subcloned into SLMAP3′. SLMAP N-terminal constructs were PCR generated using forward primer SLMAPN-F and the following reverse primers T3′ (CCTGAGTCTAGATACTTGGAGTGTTAGC; nucleotides 1-490), U3′ (CTTCTTCTAGAACATCTGTTCCCG; nucleotides 1-549) and V3′ (TGCTCTAGATCAAGCCTGCCAACTGGT; nucleotides 1-618). PCR products were each subcloned into GFP-pcDNA3 and sites of ligation were confirmed by DNA sequencing.

Immunocytochemistry

Cells grown on sterile glass coverslips were fixed by two methods. The first method involved a 10 minute exposure to 4% paraformaldehyde (PFA) in phosphate buffer at room temperature. The second method consisted of a 1 minute exposure to microtubule stabilization buffer (MTSB: 4 M glycerol, 100 mM PIPES pH 6.9, 1 mM EGTA, 5 mM $MgCl_2$), followed by a 2 minute exposure to MTSB containing 0.5% Triton-X-100 to extract soluble cellular components, then an additional 2 minute exposure to MTSB. Detergent extracted cells were then fixed with 4% PFA as described. Following either fixation method, coverslips were mounted onto glass slides and cells were incubated with relevant antibody(s) diluted in PBS containing 0.3% Triton-X-100 (PBS-T) for 3 hours at room temperature. After several washes in PBS, cells were incubated in the appropriate fluorochrome-conjugated secondary antibody(s) for 45 minutes at 37°C. DNA was stained with 4′,6-diamidino-2-phenylindole dihydrochloride (DAP1, 1 μg/ml) for 15 minutes at room temperature.

Microscopy and image analysis

Live cells were visualized with Axiovert S100 TV (Carl Zeiss) microscope equipped with SensiCam digital camera (PCO CCD Imaging). For immunocytochemistry studies, cells were visualized using Axiophot (Carl Zeiss) microscope equipped with a 3CCD colour video camera. Acquired images were digitally processed using Northern Eclipse (Version 5.0, Empix Imaging) acquisition software. Images were further processed using Adobe Photoshop™ 5.0 (Adobe Systems).

BrdU incorporation

GFP-pcDNA3 or the SLMAP expression plasmids (GFP-SLMAP3M1; GFP-SLMAP3M1ΔC; GFP-SLMAP3M2ΔC) were transiently transfected into NIH 3T3 cells grown on glass coverslips.

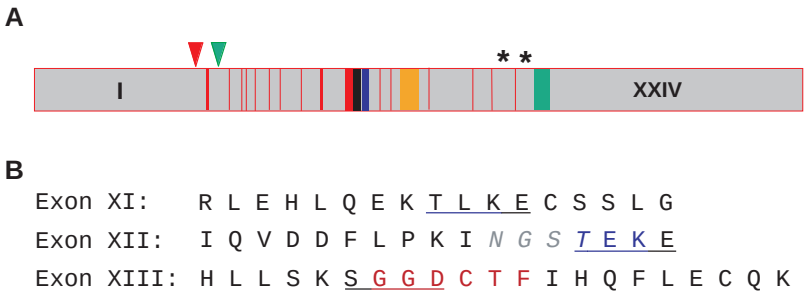


Fig. 1. Genomic organization of the SLMAP gene. (A) The SLMAP gene is composed of 24 exons and introns spanning over 122 kb of genomic DNA. Exon-exon (exons I-XXIV) boundaries are depicted. Exons XI (red), XII (black), XIII (blue), XVII (orange) and XXIII (green) encode alternative exons. Transmembrane domains are encoded by exon XXIII and exon XXIV. Exon XX and exon XXI contain the leucine zipper motifs (*). ATG1 (M1, red arrow) is encoded in exon I, whereas ATG2 (M2; green arrow) is present in exon II. (B) Amino acid composition of alternative exons XI, XII and XIII. Consensus casein kinase 2 phosphorylation sites are underlined; protein kinase C phosphorylation sites are indicated in blue; N-glycosylation site is in italics; and the N-myristolyation site is shown in red.

5′-Bromo-2′-deoxyuridine (BrdU, 10 μmol/l, Boehringer Mannheim) was added to the culture media at 36 hours post-transfection for 2, 8, 12 or 24 hours. At the end of each labeling period, cells were washed in PBS and fixed in 70% ethanol (in 50 mM glycine, pH 2) for 30 minutes at –20°C. Cells were then covered with anti-BrdU monoclonal antibody (1:10, Boehringer Mannheim) and anti-GFP rabbit antibody (1:10, Clontech) in incubation buffer (66 mM TRIS buffer, 0.66 mM $MgCl_2$, 1 mM β-mercaptoethanol) for 30 minutes at 37°C. Following several washes in PBS, cells were incubated in secondary antibodies (in PBS) for 30 minutes at 37°C. Nuclei were stained with DAP1 as previously described. Cells were then covered with mounting media and processed for immunofluorescence microscopy.

Fluorescence activated cell sorting (FACS analysis)

Transfected cells were harvested and analyzed 48 hours after removal of DNA precipitates and prepared for FACS analysis as described by Pestov et al. (Pestov et al., 1999). The DNA content of propidium iodide (Molecular Probes, 10 μg/ml) stained GFP positive cells was measured by a Becton Dickinson FACScan cytometer using an argon laser (488 nm) and analyzed with MulticycleAV (Phoenix Flow System) program.

Results

Genomic organization of the *SLMAP* gene

We previously reported that the 3′ region of the SLMAP gene is composed of 11 exons and encodes a 37 kDa SLMAP polypeptide, which is expressed in a tissue-specific manner (Wielowieyski et al., 2000). The elucidation of the genomic structure of the complete SLMAP gene may offer valuable insights into gene function. In the current study SLMAP genomic sequences were analyzed by a combination of approaches involving direct screening of mouse genomic libraries using SLMAP cDNAs, a PCR-based cloning strategy and the alignment of SLMAP cDNA with GenBank deposited genomic sequences. By direct screening of the genomic library or the PCR-based cloning strategy, eight genomic clones were isolated as outlined in Table 2. The genomic clones ranged in size from 2 kb to 20 kb. Characterization of these genomic clones revealed that the SLMAP gene spans over 122 kb of genomic DNA and consists of 24 exons (I-XXIV), including five alternative exons (XI, XII, XIII, XVII and XXIII) (Table 3, Fig. 1). Sequence data for each intron-exon boundary conformed to the canonic consensus donor (GT) and acceptor (AG) splice sites described by Mount (Mount, 1982) and is depicted in Fig. 1A. Intron sizes varied from 87 bp to over 50.5 kb of genomic DNA, whereas SLMAP exons ranged in size from 51 bp to over 1.8 kb (Table 3). Furthermore, each class of intron (0, 1 and 2) was represented within SLMAP genomic sequences (Mount, 1982).

As a result of our analysis, three additional alternative exons were identified in SLMAP. These novel alternative exons are exons XI (51 bp), XII (51 bp) and XIII (63 bp) (Table 3, Fig. 1). Each alternative exon is flanked by either class 1 introns (introns 10, 11, 13), which interrupt the coding between the first and second base of the codon, or a class 0 intron (intron 12), which occurs between codons (Mount, 1982). Notably, splicing of any of the alternative exons maintains the open reading frame of the predicted polypeptides. A PROSITE sequence scan did not predict the presence of conserved functional domains in alternative exons XI, XII or XIII; however, consensus sites for casein kinase 2 phosphorylation, protein kinase C phosphorylation, N-glycosylation and N-myristoylation were identified (Fig. 1B) (Falquet et al., 2002).

Leucine zipper motifs, two regions of specialized coiled-coil structure in the common carboxyl portion of SLMAPs, are encoded by exons XX and XXI, which correspond to exons VII and VIII in SLMAP1 (Wielowieyski et al., 2000). Two distinct transmembrane domains, TM1 and TM2, were previously identified within the extreme C-terminus of SLMAPs and are encoded by alternative exon XXIII and exon XXIV of SLMAP3, known as alternative exon X and exon XI in SLMAP1 (Wielowieyski et al., 2000). The expression of TM1 and TM2 has previously been described as mutually exclusive. Splicing of alternative exon TM1 introduces an in-frame stop codon which renders the second transmembrane domain (TM2) nonfunctional.

Two in-frame start codons (ATG1; *M*1 and ATG2; **M**2) were previously reported in the full-length cDNA sequence of the rabbit SLMAP3 isoform (Accession #U21157) (Wigle et al., 1997) and have since been found to be conserved in mouse SLMAP cDNA. Analysis of the mouse SLMAP genomic sequences revealed that the first initiating methionine (ATG1; M1) is encoded by exon I, whereas the second initiating methionine (ATG2; M2) resides in exon II. Computer-assisted sequence analysis of exon I resulted in the identification of a consensus forkhead associated domain (FHA) corresponding to amino acid 24-58 within the region spanning M1 and M2 (Fig. 2A; bold print). FHA domains exist in proteins of diverse function and have been recognized as phosphoserine/threonine-specific protein-protein interaction motifs involved

Table 3. The exon-intron junctions of SLMAP3 and sizes of the respective exons and introns

No.	Size (bp)	Exon	No.	Intron Size (bp)	Intron Class	Exon	No.
						GGCCCTTGA	I
I	1604	ACGAGCAAG**gt**aatgtcg	1	50,542	0	tcctttc**ag**TTCTATCTC	II
II	148	CCCGGAAAG**gt**atgagta	2	8847	1	tttaacc**ag**TTACCCATG	III
III	73	TCGTTCAGA**gt**gagtata	3	3571	2	actttgc**ag**TGTCATCCA	IV
IV	37	GTAGACAAA**gt**aagttgt	4	1818	0	tatttct**ag**GTTGCTGCT	V
V	63	TATCTACAG**gt**aaaagtc	5	2819	0	ttatgtt**ag**GAGGCCTTA	VI
VI	96	AGTTGGCAG**gt**attccat	6	295	0	tttcaac**ag**GCTTTAATA	VII
VII	72	TGCTCCAAA**gt**aggtatt	7	1484	0	ttatcct**ag**AATCAAACA	VIII
VIII	141	GAAGTTGAG**gt**atttcac	8	1178	0	gccttgc**ag**CGAAGTCTG	IX
IX	138	AAATTAAAG**gt**atgtata	9	2050	0	atgcaac**ag**GTAGCAGAG	X
X	169	CTCTGCAAG**gt**aagctgg	10	87	1	tttttac**ag**TACGGTTAG	XI*
XI	51	GCAGCTTAG**gt**aggtgtc	11	443	1	taatcct**ag**GGATACAAG	XII*
XII	51	CAGAAAAAG**gta**gcgtaa	12	5809	0	ctttatt**ag**AGCACTTGC	XIII*
XIII	63	AGTGCCAGA**gt**gagtaca	13	10,903	1	ctttggc**ag**AGAAGCTGA	XIV
XIV	60	TTTCAAAAG**gt**tagtttg	14	3546	1	tcctaat**ag**AAAATCAGG	XV
XV	81	ACACTACAG**gt**gagtgcg	15	290	1	cccttat**ag**ACGCCCAGA	XVI
XVI	60	TATTAAAAG**gt**attttaa	16	8768	1	ttttgtc**ag**ATGACTTAC	XVII*
XVII	123	AACTTCAAG**gt**gagatga	17	1471	1	gcttttt**ag**CTCTTTTGG	XVIII
XVIII	75	TTCTTCAAG**gt**atggggc	18	686	1	cttttac**ag**TCCAGCTGC	XIX
XIX	321	GGTTGCAAG**gt**aagtgag	19	552	1	ctactttag**GTGAGCTGG**	XX
XX	118	ATTGCACAA**gt**atgtgag	20	3974	2	gtgttgc**ag**TTCTCAGAA	XXI
XXI	172	CAGAAAGAG**gt**aaagcgg	21	4243	0	tgcctttag**TATGAAAAG**	XXII
XXII	135	GGAAATAAT**gt**aagtgtt	22	1820	0	cttcttc**ag**CCCTCCATA	XXIII*
XXIII	90	TAGAGAAAG**gt**acaagca	23	1214	0	tccacac**ag**AAACCCTGG	XXIV
XXIV	1832	TAATCTCCC					

*Alternative exon.
Exon-intron boundaries conform to the consensus splice donor and acceptor sites (gt-ag).

in phosphopeptide recognition (Durocher et al., 2000; Sun et al., 1998).

Endogenous expression and subcellular distribution of SLMAP

In vitro transcription-translation experiments revealed the presence of two SLMAP products whose molecular masses were similar to those predicted by the utilization of M1 (91 kDa) or M2 (80 kDa) (Wigle et al., 1997). To test whether a 91 kDa protein is produced in vivo, implying the utilization of M1 as the initiating methionine, antibodies were generated against a peptide sequence in the M1-M2 region at the N-terminus of SLMAP (anti-SLMAP-N) or against a fusion protein encompassing the C-terminal region of SLMAP (anti-SLMAP-C). Both antibodies recognized a 91 kDa protein in NIH 3T3 cell extracts (Fig. 2B, lanes 1 and 2). Immunoadsorption of the anti-SLMAP(N) antibodies with purified SLMAP fusion protein confirmed the specificity of the antiserum (Fig. 2B, lane 3). These results suggest that a 91 kDa polypeptide is produced from M1 of SLMAP3 mRNA in NIH 3T3 cells.

The use of M1 as a translation initiation codon is predicted to add 132 residues to the N-terminal region of the previously identified SLMAP3 isoform. We then examined if these residues confer different biochemical and subcellular properties to SLMAP3M1 not previously described for other SLMAP isoforms. Immunocytochemical analysis of NIH 3T3

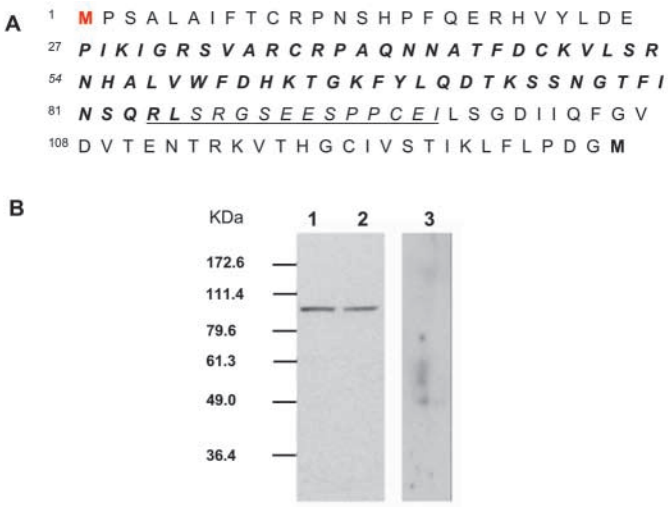
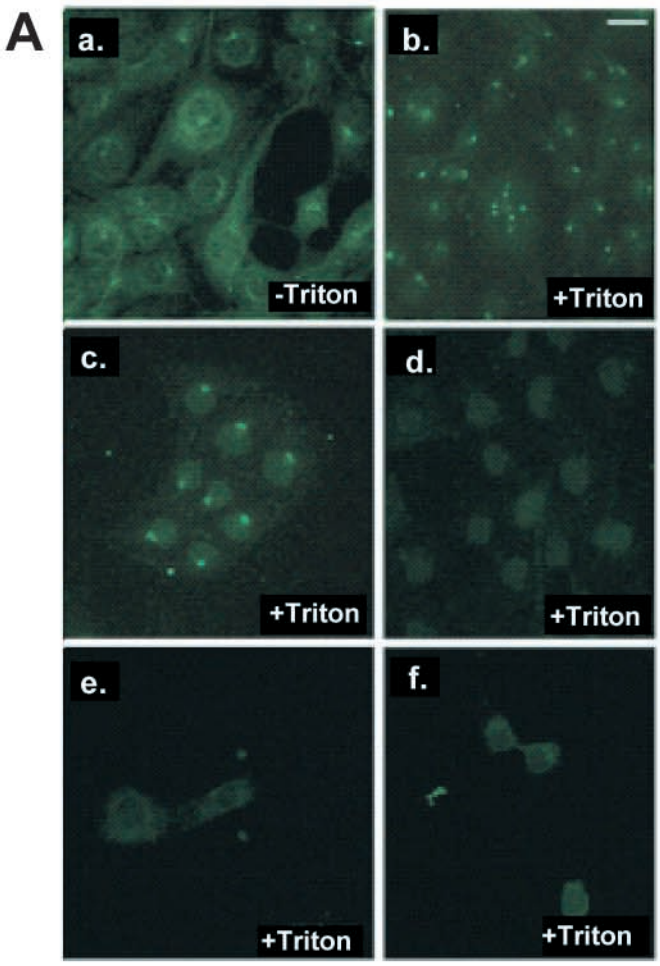


Fig. 2. SLMAP3M1 is characterized by a unique N-terminal extension. (A) Amino acid composition of the N-terminal extension identified in SLMAP3. M1 (red) and M2 (blue) represent the two initiating methionines used to generate SLMAPs (Wigle et al., 1997). The consensus fork head associated (FHA) domain is demarcated by italics in bold print. Peptide sequence used to generate anti-SLMAP(N) rabbit antiserum is underlined (RLSRGSEESPPCEI). (B) Immunoblot analysis of SLMAP expression in NIH 3T3 cells. NIH 3T3 cells were lysed in RIPA buffer; 15 μg of total protein was separated by 10% SDS-PAGE then electrotransferred to PVDF membranes. Immunoblotting with anti-SLMAP(C) antibody (lane 1) or anti-SLMAP(N) antisera (lane 2) detected a single band at 91 kDa. Prior incubation of the anti-SLMAP(N) with SLMAP peptide abolished the immunoreactivity (lane 3). Positions of molecular mass standards are indicated (BENCHMARK Prestained Protein ladder, Gibco BRL).

cells that exclusively express the 91 kDa isoform revealed that SLMAP3M1 resides in several subcellular compartments. While diffuse cytoplasmic SLMAP staining was evident, SLMAP proteins could also be detected in one or two foci generally situated adjacent to the nuclear membrane (Fig. 3Aa,b). The foci staining pattern, but not the diffuse cytoplasmic staining pattern, was resistant to detergent extraction, suggesting that SLMAP3M1 is tightly assembled with detergent-insoluble structures (Fig. 3Ab). The N-terminal directed peptide antibody (anti-SLMAP(N); Fig. 3Ac), but not the control pre-immune serum (Fig. 3Ad), detected SLMAP proteins at perinuclear foci in detergent extracted COS-7 cells as well. Peptide competition experiments abolished the staining pattern observed with anti-SLMAP(C) (Fig. 3Ae) and incubation with the pre-immune antisera also abolished staining (Fig. 3Af), thus confirming the antibody specificity for SLMAP proteins. The subcellular distribution of SLMAP was similar in other cells that express the 91 kDa SLMAP isoform, including



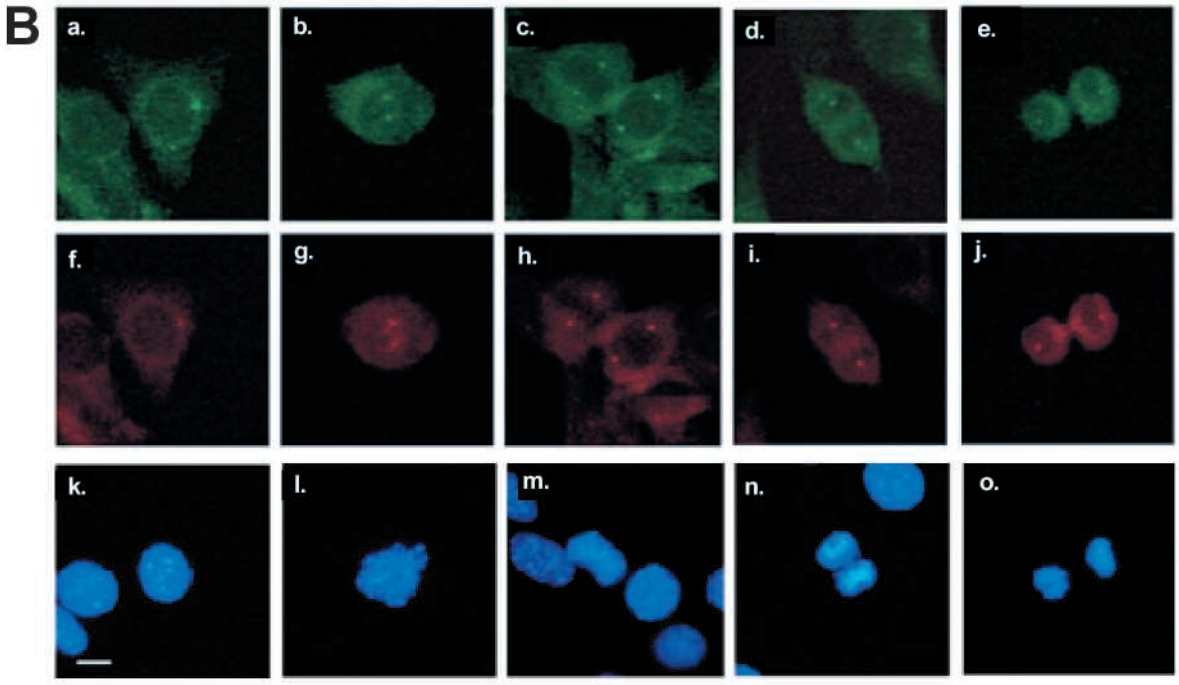


Fig. 3. Subcellular localization of endogenous SLMAP in NIH 3T3 cells. (A) Immunofluorescent labeling of SLMAP proteins using anti-SLMAP(C) antiserum revealed staining of reticular formations, the cytosol and perinuclear sites in paraformaldehyde fixed NIH 3T3 cells (a). Reduced cytosolic SLMAP staining and labeling of distinct foci at the perinuclear region was observed in detergent-extracted, paraformaldehyde-fixed cells (b). No immunoreactivity was observed with prior incubation of SLMAP(C) antisera with purified SLMAP protein (e) or in cells incubated with the pre-immune rabbit serum (f). Perinuclear foci staining was also evident in detergent extracted COS-7 cells stained with anti-SLMAP(N) (c). This staining pattern was not observed in COS-7 cells stained with the pre-immune serum (d). (B) SLMAP colocalizes with γ-tubulin at centrosomes throughout the cell cycle. Paraformaldehyde-fixed NIH 3T3 cells were co-stained with anti-SLMAP (a-e), anti-γ-tubulin (f-j) and DAP1 (k-o). Representative cells in interphase (a,f,k), prophase (b,g,l), metaphase (c,h,m), anaphase (d,i,n) and telophase (e,j,o) are shown. Bars, 20 μm.

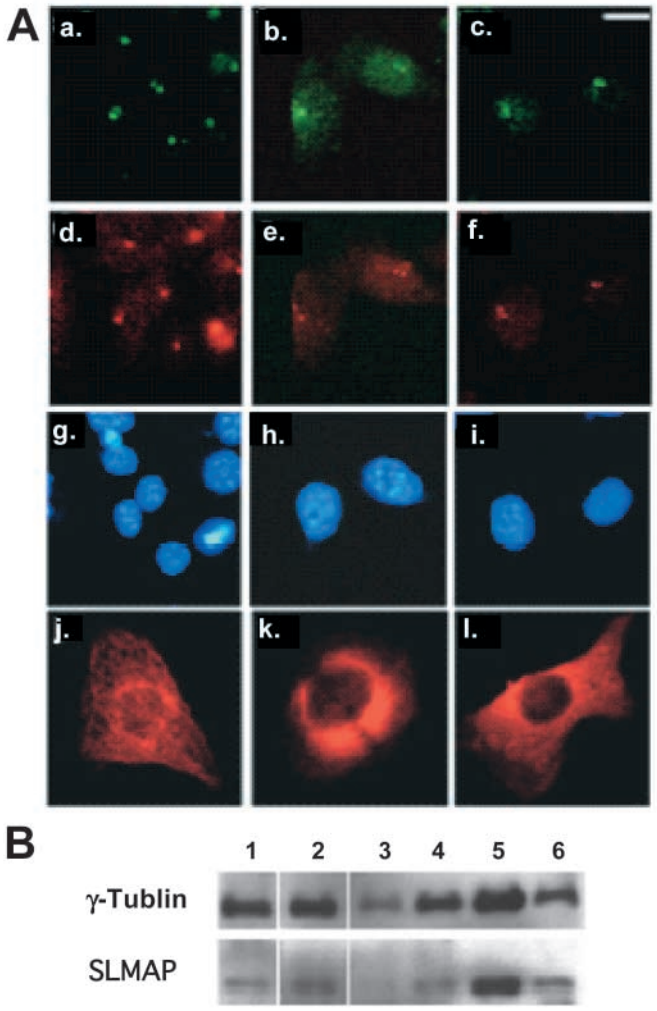


Fig. 4. SLMAP is a component of centrosomes. (A) SLMAP localization at centrosomes is not affected by nocodazole or taxol treatment. NIH 3T3 cells were untreated (a,d,g,j) or treated with either taxol (5 μM, 4 hours; b,e,h,k) to stabilize microtubules or nocodazole (6 μg/ml, 4 hours; c,f,i,l) to depolymerize microtubules. Cells were prepared for immunofluorescence microscopy and co-stained with anti-SLMAP (a,b,c), anti-γ-tubulin (d,e,f) and DAP1 (g,h,i). To show that the drugs affected cytoplasmic microtubules, cells were stained with anti-α-tubulin monoclonal antibodies (j,k,l). Bar, 25 μm. (B) Centrosomes were isolated from exponentially growing NIH 3T3 cells by fractionation on a sucrose gradient as described previously (Moudjou and Bornens, 1998). Equivalent amount (15 μg) of each protein fraction was analyzed by SDS-PAGE followed by western blotting with anti-SLMAP or anti-γ-tubulin. Lane 1 (lysed extract), lane 2 (crude centrosomes) and lanes 3-6 represent fractions obtained from sucrose density gradients.

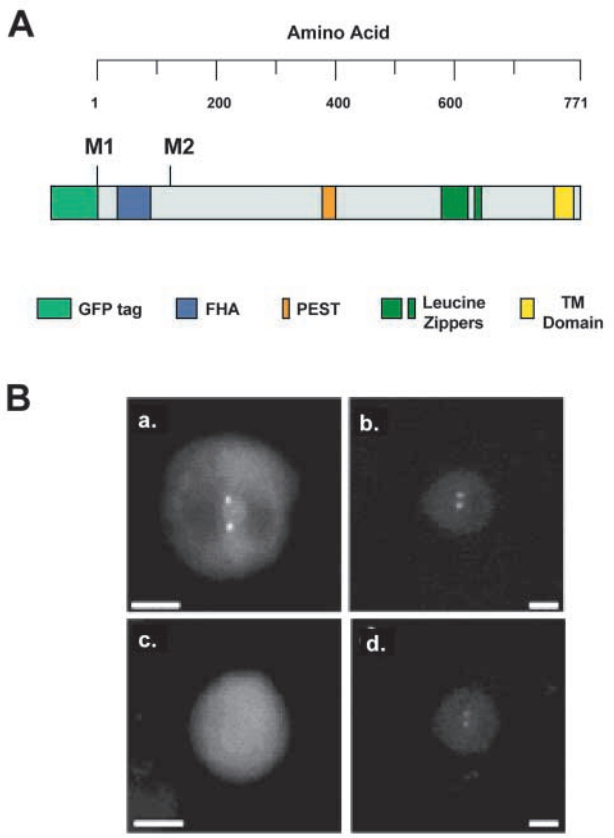


Fig. 5. SLMAP3M1 sequences target GFP to centrosomes. (A) GFP-SLMAPM1 fusion protein used for transient transfection studies. Depicted are the GFP tag, the FHA domain, PEST motif, leucine zippers and the transmembrane (TM) domain. (B) Localization of GFP-SLMAP fusion proteins transiently expressed in NIH 3T3 cells was visualized by live microscopy. GFP fluorescence was detected at centrosomes in mitotic cells expressing GFP-SLMAP3M1 (a, two closely spaced dots). No green fluorescence was observed at centrosomes in control mitotic cells transfected with GFP-pcDNA alone (c). Co-incidence of the γ-tubulin signal (d) and GFP fluorescence (b) occurred in paraformaldehyde fixed mitotic cells expressing GFP-SLMAP3M1. Bar, 25 μm.

murine myoblasts (C2C12) and embryonic stem cells (data not shown).

SLMAP is a novel component of the centrosome

The perinuclear foci staining of SLMAP is reminiscent of proteins that localize to the centrosome. To determine if SLMAP is associated with centrosomes, colocalization experiments were performed with γ-tubulin, a well-characterized core centrosomal component (Stearns et al., 1991). As illustrated in Fig. 3B, SLMAP labeling was co-incident with the γ-tubulin signal at each stage of the cell cycle as determined by DAPI staining. Interphase (G1) cells (Fig. 3Ba,f,k) contained one centrosome stained by both anti-SLMAP and anti-γ-tubulin. In mitotic cells SLMAP (Fig. 3Bb-e) colocalized with γ-tubulin (Fig. 3Bg-j) from prophase to anaphase (Fig. 3Bl-o). The SLMAP protein appears to have a symmetrical distribution during centrosome division given that anti-SLMAP appeared to stain each centrosome with equal intensity.

Many proteins reside at the minus ends of microtubules and are thus dependent on microtubules for their associations with the MTOC. These proteins can be distinguished from integral centrosomal proteins by treatments with agents that specifically disrupt microtubules. The fungal toxin nocodazole causes depolymerization of the cytoplasmic microtubule network, whereas taxol (paclitaxel) acts to stabilize cytoplasmic microtubules by binding tubulin directly along the

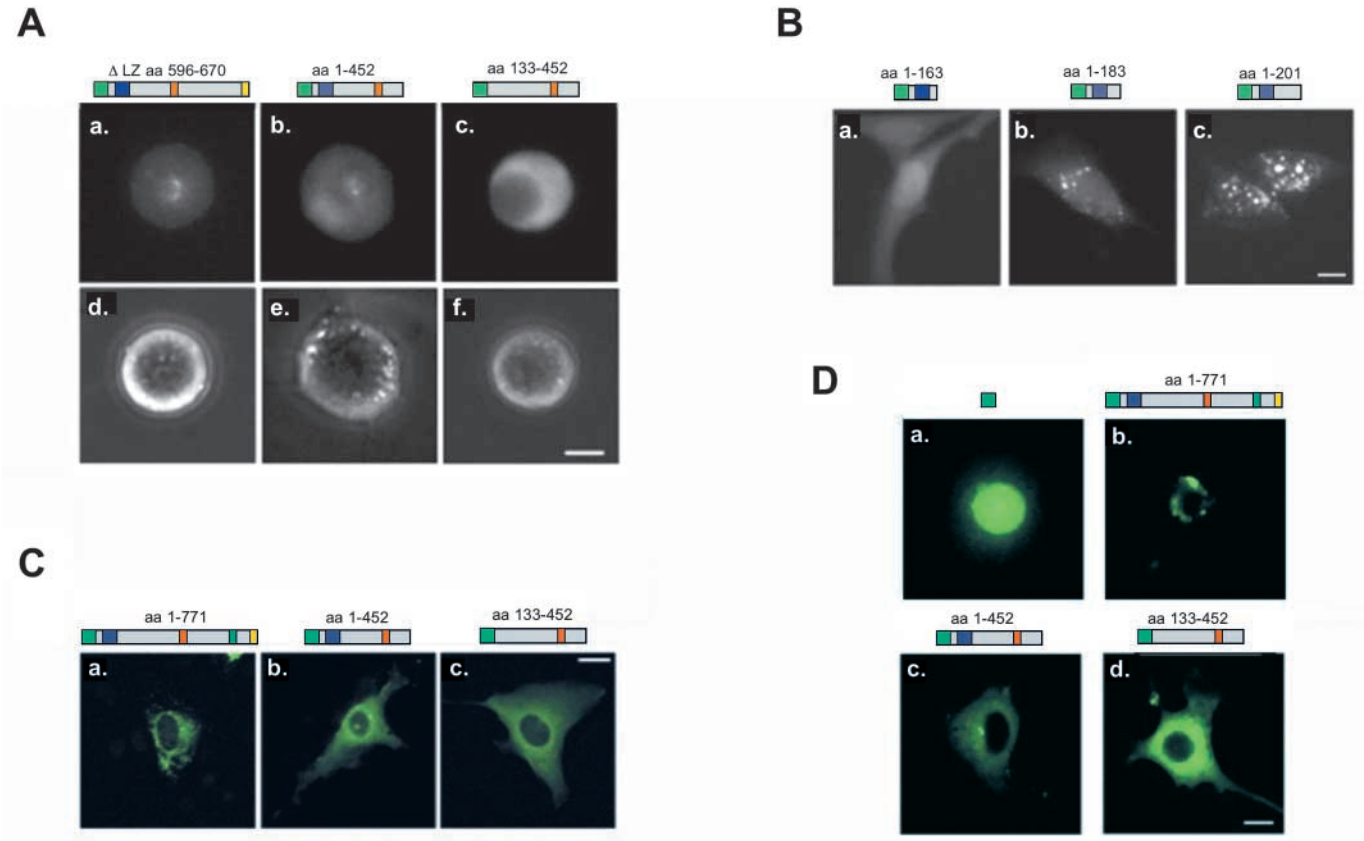


Fig. 6. N-terminal sequences of SLMAP are required for targeting GFP to centrosomes. (A) Transient expression of GFP-tagged SLMAP mutants showed that green fluorescence was observed at centrosomes in live cells expressing GFP-SLMAP3M1ΔLZ (a) and GFP-SLMAP3M1ΔC (b) but not in mitotic cells expressing GFP-SLMAP3M2ΔC (c). Corresponding phase contrast images are shown for SLMAP3M1ΔLZ (d), GFP-SLAP3M1ΔC (e) and GFP-SLMAP3M2ΔC (f). Bar, 25 μm. (B) N-terminal SLMAP3 constructs encoding amino acids 1-163 (a), 1-183 (b) or 1-206 (c) were expressed in fibroblast cells. GFP fluorescence was detected throughout the cell, including the nucleus in (a) or within detergent-insoluble aggregates (b, c). Bar, 10 μm. (C) Transient expression of the full-length GFP-SLMAP fusion protein, GFP-SLMAP3M1 (a), was detected at perinuclear sites in interphase cells, indicative of membrane localization. Diffuse cytosolic localization was observed for both C-terminal mutants, GFP-SLMAP3M1ΔC (b) and SLMAP3M2ΔC (c). Bar, 50 μm. (D) At 36 hours post-transfection, cells expressing GFP-SLMAP3M1 were rounded (b), whereas those cells expressing either GFP-pcDNA3 (a), GFP-SLMAP3M1ΔC (c) or GFP-SLMAP3M2ΔC appeared normal. Bar, 10 μm.

length of the microtubule (De Brabander et al., 1986). On treatment with either drug, integral centrosomal proteins remain bound to centrosomal components, whereas microtubule-dependent centrosome-associated proteins are no longer retained at the MTOC (De Brabander et al., 1986). Under control conditions (untreated) SLMAP3M1 was found to colocalize with γ-tubulin (Fig. 4Aa,d). The effects of taxol (Fig. 4Ak) and nocodazole (Fig. 4Al) on microtubules were confirmed by staining with α-tubulin, which illustrated a distinct redistribution of cytoplasmic microtubules. Treatment with taxol (Fig. 4Ab,e,h,k) or nocodazole (Fig. 4Ac,f,i,l) did not alter the localization of SLMAPs at centrosomes. These findings confirm that SLMAP localization at the centrosome is independent of microtubule assembly.

To show biochemically that SLMAP is a component of centrosomes, these subcellular structures were purified from NIH 3T3 cell extracts by fractionation on a sucrose density gradient (Moudjou and Bornens, 1998). The sucrose gradient fractions were resolved by SDS-PAGE and examined by immunoblotting with anti-γ-tubulin and anti-SLMAP (Fig. 4B). The centrosomal marker γ-tubulin was present in the cell lysate (lane 1), enriched in the crude centrosome preparation (lane 2) and even further concentrated in fraction 2 (lane 5) from the sucrose density gradient. The 91 kDa SLMAP3M1 protein was found to co-enrich with the γ-tubulin peak in fraction 2 (lane 5) of the purified centrosome preparation.

Centrosomal targeting is mediated by the novel N-terminal region of SLMAP

The data presented indicates that SLMAP3M1 is an integral component of centrosomes. These observations led us to hypothesize that the newly identified sequences from the M1 initiating codon might play a role in targeting SLMAP to the MTOC. To test this hypothesis *SLMAP3M1* cDNA (Fig. 5A) and a series of *SLMAP* deletion mutants (Fig. 6A-D) were fused in frame to GFP to produce GFP-SLMAP fusion proteins. The GFP-SLMAP fusion constructs were used for transient expression studies in NIH 3T3 cells. Fig. 5B (a) shows that SLMAP3M1 cDNA encodes information that is sufficient to target the heterologous reporter GFP to the centrosome in live cells. This localization pattern was not

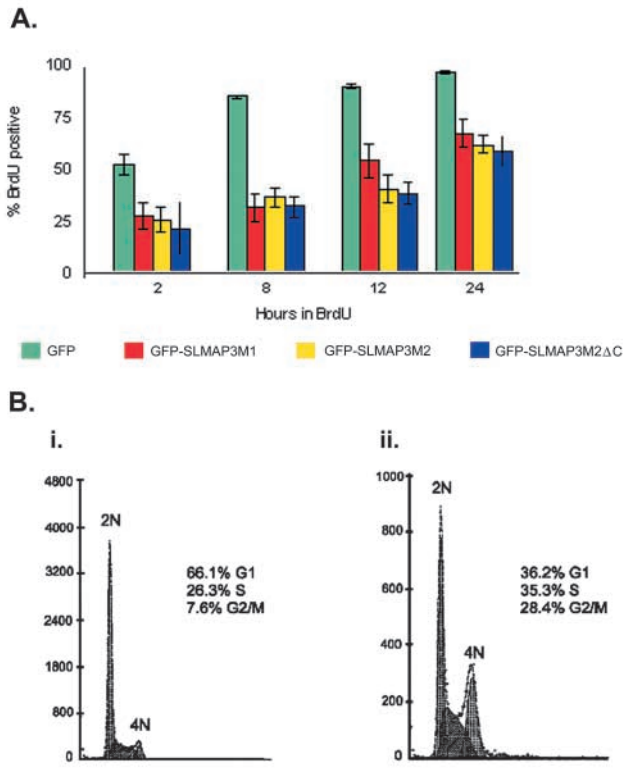


Fig. 7. SLMAP overexpression affects cell proliferation. (A) Overexpression of GFP-tagged SLMAPs inhibits BrdU uptake in NIH 3T3 cells. NIH 3T3 cells were transfected with either GFPpcDNA3 (green bar), GFP-SLMAP3M1 (red bar), GFP-SLMAP3M2 (yellow bar) or GFP-SLMAP3M2ΔC (blue bar). Thirty-six hours post-transfection, cells were labeled with BrdU (100 mmol/l) for 2, 8, 12 or 24 hours. Ethanol fixed cells were co-stained with anti-mouse BrdU antibody and anti-rabbit GFP antibody. Transfected cells were identified by their green fluorescence and the number of cells with co-existing nuclear red fluorescence (BrdU positive cells) was determined. The mean results of at least three independent experiments for each labeling period are shown. For each transfectant and condition, a minimum of 200 cells were scored. Error bars represent standard deviations. (B) FACS profiles of GFP-pcDNA3 (i) and GFP-SLMAP3M2 (ii) transfected NIH 3T3 cells. At 48 hours post-transfection, cells were incubated with propidium iodide to stain DNA (Pestov et al., 1999).

observed in live mitotic cells expressing GFP alone (Fig. 5Bc). GFP-SLMAP3M1 colocalized with γ-tubulin at the centrosome (Fig. 5Bb,d), consistent with the localization of endogenous SLMAP3M1 (Figs 3, 4). Deletion of the two leucine zipper regions did not affect GFP targeting to the centrosome (Fig. 6Aa), and the C-terminal truncation mutant (SLMAP3M1ΔC) also retained the ability to target GFP to centrosomes (Fig. 6Ab). A cDNA encoding SLMAP initiated from the second methionine (GFP-SLMAP3M2ΔC) lacking the transmembrane domain was unable to target GFP to centrosomes (Fig. 6Ac). It is notable that C-terminal mutants lacking the putative transmembrane domain resulted in the exclusion of SLMAP from the perinuclear membranes and reticular formations (Fig. 6Cb,c), which is consistent with the observation that C-terminal sequences play a role in membrane targeting of SLMAP (Wielowieyski et al., 2000). These results suggest that the newly described N-terminal sequences encompassing the FHA domain of SLMAP contain information required for its centrosomal localization.

To identify the minimal sequences present in SLMAP sufficient for targeting to the MTOC, a series of SLMAP N-terminal fragments fused to GFP were utilized in transient expression studies in NIH 3T3 fibroblast cells. Diffuse cytosolic and nuclear localization was observed in cells expressing the GFP-SLMAP mutant expressing amino acids 1-163 (Fig. 6Ba), while GFP-positive detergent-insoluble aggregates were detected in cells transfected with SLMAP mutants expressing amino acids 1-183 and 1-206 (Fig. 6Bb,c). On the basis of observations by live microscopy and co-staining with the centrosome marker protein γ-tubulin (data not shown), GFP fluorescence was not detected at centrosomes in cells overexpressing these extreme N-terminal SLMAP3M1 mutants. These observations suggest that N-terminal sequences, in addition to preservation of the overall structure of SLMAP or SLMAP-mediated protein interactions, may be important determinants for targeting SLMAPs to centrosomes.

SLMAP overexpression affects cell proliferation

The finding that SLMAP3M1 was localized at the centrosome raised the possibility as to whether SLMAPs serve a role in mitosis and cell growth, and we examined this by ectopic expression studies. We observed that a large proportion of cells expressing the full-length GFP-SLMAP3M1 were nonviable and rounded in appearance (Fig. 6Db) and detached from the coverslips within 36 hours following transfection. By contrast, cell viability did not appear to be affected by the expression of either the C-terminal mutant (GFP-SLMAP3M1ΔC; Fig. 6Dc) or the GFP-tagged SLMAP mutant lacking both the N- and C-terminal sequences (GFP-SLMAP3M2ΔC; Fig. 6Dd). To address this issue further we examined the effect of ectopic expression of SLMAP3M1, SLMAP3M2 and SLMAP3M2ΔC on cell proliferation. A reduction in the level of BrdU incorporation by more than twofold was observed in cells expressing GFP-SLMAP3M1 fusion protein relative to GFP transfected control cells for labeling periods of 2 to 8 hours (Fig. 7A). Approximately 60% of cells expressing either SLMAP3M1, SLMAP3M2 or SLMAP3M2ΔC displayed BrdU incorporation after a 24 hour pulse, whereas essentially all of the control cells were positive for BrdU. These results indicate that regulated levels of SLMAPs are important for normal cell growth.

We sought to determine whether overexpression of SLMAPs altered cell proliferation by interfering in a cell cycle phase-specific manner. Fluorescence activated cell sorting (FACS) was used to identify GFP-expressing cells and thereby identify changes in cell cycle progression induced by SLMAP (Fig. 7B). Overproduction of GFP-SLMAP3M1 was toxic to NIH 3T3 cells as the majority of cells died within 36 hours post-transfection. However, cells expressing the deletion mutant GFP-SLMAP3M2 remained viable for the time required for FACS analysis, and overproduction of this deletion mutant caused about a fourfold increase in G2/M (4N DNA; Fig. 7Bii) content compared with control cells (Fig. 7Bi). These results suggest that deregulated levels of SLMAP3 in NIH 3T3 cells alter proliferation by interfering at the G2/M phase of the cell cycle.

Discussion

In the present study, we show that a novel SLMAP isoform is a component of the MTOC and may serve a role in centrosomal functions. The SLMAP gene is encoded by 24 exons that span over ~122 kb of continuous DNA sequence. Analysis of the new sequences defined the presence of three additional regions of alternative splicing (exons XI, XII, XIII) in *SLMAP* compared with our previous results. A new SLMAP variant (SLMAP3M1) containing an N-terminal extension of 132 amino acid residues that are encoded in exon I was identified to be a component of the MTOC. Using anti-peptide antibodies directed toward the N-terminal sequences, a SLMAP protein of ~91 kDa was identified in various cell extracts. These findings confirm that SLMAP3M1 represents a ubiquitously expressed SLMAP isoform. Indirect immunofluorescence studies using fixation procedures known to aid visualization of the cytoskeleton localized SLMAP3M1 to the centrosome. SLMAP3M1 was detected at the centrosome at all stages of the cell cycle (therefore not a transient association) and colocalized with γ-tubulin. Treatments with reagents that specifically disrupt cytoplasmic microtubules did not affect SLMAP association at centrosomes, indicating that SLMAP-centrosome association occurs independently of microtubules. This new SLMAP isoform may be a core component of the MTOC.

SLMAP3M1 sequences were found to contain the information required to target the heterologous protein GFP to centrosomes. Large C-terminal deletions eliminating much of the coiled-coil structure including the leucine zipper motifs did not affect targeting of GFP to centrosomes. However, removal of the newly identified N-terminal residues from SLMAP abolished the targeting of GFP to the centrosome, indicating that the 132 N-terminal amino acid residues are required for centrosomal targeting. Further analysis of SLMAP deletion mutants encoding N-terminal sequences revealed that this region of SLMAP is not alone sufficient for centrosome targeting. Although the N-terminus of SLMAP may serve a crucial role in mediating SLMAP-centrosome associations, the preservation of the overall structure of SLMAP may constitute an additional determinant for ensuring faithful targeting at the MTOC. Conserved motifs for protein targeting to and retention within organelles such as the ER and Golgi have been characterized (Pelham, 1988; Jackson et al., 1990; Kjer-Nielsen et al., 1999; Munro and Nichols, 1999; Brown et al., 2001), yet little is known of conserved sequences directing proteins to centrosomes. Recent studies have highlighted a conserved region of approximately 90 amino acids encoded in pericentrin and AKAP450 that confers targeting to centrosomes (Gillingham and Munro, 2000). This motif, known as the PACT domain, was not identified within the N-terminal sequences of SLMAP or within any other region of this molecule. Thus, our results indicate that structural motifs, in addition to motifs such as the PACT domain, are crucial in targeting components to centrosomes. In this regard, recent studies by Petit et al. (Petit et al., 2003) indicate that coiled-coil motifs play important roles in targeting molecules to the centrosome.

Amino acid sequence analysis of the new SLMAP N-terminal revealed the presence of a conserved motif known as the forkhead associated (FHA) domain. FHA domains have been characterized in several proteins of diverse function, including kinases, phosphatases, kinesins, transcription factors, and DNA- and RNA-binding proteins, as well as metabolic enzymes (Hofmann and Bucher, 1995; Durocher and Jackson, 2002), and are known to serve as phosphoserine/threonine-specific protein-protein interaction motifs (Durocher et al., 2000; Sun et al., 1998). In this regard, FHA-interacting proteins such as Hklp2, a human homolog of Xklp2, a Xenopus kinesin-like motor protein, which serve roles in centrosome separation and spindle bipolarity, are known to reside at the MTOC (Boleti et al., 1996; Sueishi et al., 2000). The FHA domain in SLMAP may thus mediate associations with phosphoproteins present at MTOC. In this regard, the Aurora family of mitotic serine-threonine kinases is required for MTOC functions (Kimura et al., 1997; Kimura et al., 1999; Giet and Prigent, 1999). The presence of the FHA domains in SLMAP raises the possibility that it may interact with phosphorylated substrates of this kinase to affect centrosome activity and cell viability.

SLMAP shares several features inherent to other centrosomal proteins, including a net negative charge (pI 4.9) and extensive regions of coiled-coil structure (Doxsey et al., 1994; Boukson-Castaing et al., 1996; Errabolu et al., 1994). The alternative exons XI, XII and XIII were predicted to introduce post-translational modification sites including phosphorylation for casein kinase 2 and protein kinase C, as well as N-glycosylation and N-myristoylation. Such modifications may impose significant consequences with respect to the biological function and regulation of SLMAPs. In view of the extensive splicing predicted in SLMAP, we speculate that alternative splicing may represent a fundamental mechanism that confers functional diversity among SLMAP variants as has been reported for other coiled-coil proteins. Our recent unpublished data indicate that SLMAP isoforms can homodimerize via the specialized leucine-rich coiled-coil regions and reside in different cellular organelles, including the Golgi apparatus. In this regard the SLMAPs are similar to Golgin 97 and TGN 38, which can also reside in the Golgi and the centrosome (Takatsuki et al., 2002). Studies indicate that the spatial arrangement of the Golgi with respect to the centrosome is important for Golgi organization following mitosis, and tethering these two organelles may be a critical event mediated by as-yet-unidentified molecules (Sutterlin et al., 2002; Takatsuki et al., 2002). The SLMAPs share an overall structural homology with docking molecules such as the syntaxins and may therefore contribute to tethering function for the centrosome and the Golgi, possibly via homodimer formation. Although we do not know the precise function of SLMAP at centrosomes, the deregulation of SLMAP expression had a marked effect on cell viability and cell cycle progression. It is notable that recent data has implicated a crucial role for the centrosome in cell cycle control, although the identity of the molecular components involved remain to be defined (Rieder et al., 2001). Overall, our findings imply that the new SLMAP isoform is a component of the centrosome whose targeting is mediated by its unique structural determinants and as such may participate in the important functional roles being ascribed to this organelle.

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