Janmp1 (Marlin-1) Defines a Family of Proteins Interacting with Janus Kinases and Microtubules*

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Janmp1 (Jak and microtubule interacting protein), an alias of Marlin-1, was identified for its ability to bind to the FERM (band 4.1 ezrin/radixin/moesin) homology domain of Tyk2, a member of the Janus kinase (Jak) family of non-receptor tyrosine kinases that are central elements of cytokine signaling cascades. Jamip1 belongs to a family of three genes conserved in vertebrates and is predominantly expressed in neural tissues and lymphoid organs. Jamip proteins lack known domains and are extremely rich in predicted coiled coils that mediate dimerization. In our initial characterization of Jamip1 (73 kDa), we found that it comprises an N-terminal region that targets the protein to microtubule polymers and, when overexpressed in fibroblasts, profoundly perturbs the microtubule network, inducing the formation of tight and stable bundles. Jamip1 was shown to associate with two Jak family members, Tyk2 and Jak1, in Jurkat T cells via its C-terminal region. The restricted expression of Jamip1 and its ability to associate to and modify microtubule polymers suggest a specialized function of these proteins in dynamic processes, e.g. cell polarization, segregation of signaling complexes, and vesicle traffic, some of which may involve Jak tyrosine kinases.

The four Jak proteins (Tyk2 and Jak1–3) are 125–140 kDa non-receptor tyrosine kinases that were first identified in mammalian cells for their involvement in signaling via a distinct family of plasma membrane complexes, i.e. the cytokine receptors. These receptors bind cytokines with a common α-helical bundled folding and utilize Jak proteins as their catalytic moieties in a way that recalls the functioning of receptor tyrosine kinases (1). Since the discovery of the Jak proteins a decade ago much work has been done, ranging from the study of their structure/function organization, post-translational modifications, and subcellular localization to the analysis of knock-out animals and the identification of mutations and translocations responsible for immune deficiency or cancer (2, 3). These studies have validated the essential role of Jak enzymes in cytokine signaling and helped in understanding their specificity of action.

Jaks contain an N-terminal FERM (band 4.1 ezrin/radixin/moesin) homology domain, a putative Src homology 2 domain, a regulatory kinase-like domain, and a tyrosine kinase domain activated by tyrosine phosphorylation (1). Although structure/function analyses suggest a high flexibility and communication among the various parts of the molecule, no crystallographic data are yet available for any of these modular domains. The three-dimensional structures of the moesin and radixin FERM domains were shown to comprise three lobes with similarities to ubiquitin, the acyl-CoA-binding protein, and pleckstrin homology/phosphotyrosine binding-like domains (4, 5). In Jak proteins, the FERM domain is involved in an ill-defined, non-covalent interaction with the membrane-proximal region of cytokine receptors, and, as proposed for the ezrin/radixin/moesin proteins, it may act as a compact architectural unit with multiple binding surfaces. Jaks were reported to bind to a variety of signaling proteins such as SHP1/2, Syk, PP2A, P3K, Fyn, Yes, Shc, Vav, and Cbl (1). Other Jak interactors were identified by yeast two-hybrid screens, namely Src1 (6), Stat5 (7), and JBP1/PRMT5, a protein methyltransferase involved in growth control (8). Some of these complexes were shown to form in a phosphorylation-dependent manner.

Recent findings suggest that the binding of Jaks to cytokine receptors may occur early during biosynthesis at the level of endoplasmic reticulum or Golgi membranes, as Jaks were found to potentiate the maturation of at least some receptors (9, 10). One family member, Tyk2, was found to stabilize a cognate receptor at the plasma membrane by reducing its rate of internalization (11). Another family member, Jak2, was identified as a constituent of the transitional endoplasmic reticulum (12). Thus, the possibility is emerging that Jaks may not simply act as catalytic moieties of cytokine receptors but may be engaged in interactions with cellular constituents that affect protein assembly and transport processes.

Based on these premises, we set out to identify new interactors of Tyk2 using its isolated FERM domain as bait in a two-hybrid screen of a Jurkat T cell line cDNA library. Here, we report on the identification and initial characterization of a protein, which we have designated Jamip1 (acronym of Jak and microtubule interacting protein), that defines a new class of proteins rich in coiled coils and associated with the microtubule.
Interactions of Jamip Proteins with Jak and Microtubules

### EXPERIMENTAL PROCEDURES

#### Yeast Two-hybrid Screen—The FERM domain of human Tyk2 (aa 1–451) was cloned in pGBT9 and used as bait in the HP7 yeast strain transformed with a human Jurkat cDNA library in the pACT2 vector (Clontech). 10⁶ transformants were plated on medium lacking histidine. Colonies were then tested for β-galactosidase activity. Transformants were cured of the bait plasmid and re-tested for specific interaction. Yeast plasmid DNA was isolated, rescued into Escherichia coli XL-1, re-transformed into the bait-containing yeast strain, and assayed again.

**Plasmid Constructs**—The full-length Jamip1 coding sequence was obtained by reverse PCR using as template human brain cDNA (Clontech) and cloned in pcDNA3.1/V5-His/neo (Invitrogen) and p3XFLAG-CMV (Sigma). The N-ter sequence was obtained by PCR using as template pcDNA3-Jamip1. The C-ter sequence, obtained by PCR using as template the yeast clone, retains the original HA tag. Both PCR products were subcloned in pcDNA4/V5-His/neo (Invitrogen). To assay tyrosine phosphorylation (Fig. 5A), we used a C-ter expression plasmid in which the HA tag was removed by deleting a HindIII-BamHI fragment. Jamip2 was obtained by subcloning the amplification product as a SalI-AflII fragment into pET28a+ (Novagen). FLAG-Jak1 was obtained by subcloning the amplified human Jak kinases and the dynamic microtubule network.

**Detergent Extraction Assay**—Adherent cells were washed in PHEM (45 mM Pipes, pH 6.8, 45 mM Heps, pH 6.8, 5 mM MgCl₂, and 10 mM EGTA) and extracted for 5 min at room temperature with 400 μl of 0.5% Triton X-100 in PHEM and protease inhibitors. The supernatant (soluble) was collected. The detergent-insoluble matrix remaining on the dish was washed in PHEM, extracted in 400 μl of Laemmli buffer, scraped, and harvested (insoluble). For cells pretreated with drugs, all washes were added to the drug. Equal volumes of soluble and insoluble fractions were loaded on SDS-PAGE gels. Proteins were detected by western blot. 5 × 10⁶ Jurkat cells (5 × 10⁶/μl) were treated with drugs, pelleted, transferred to 2-ml tubes, and extracted as described above, except that the soluble fraction was collected by centrifugation at 700 × g for 5 min.

**Fluorescent Microscopy**—HT-1080 cells were transfected on glass coverslips with 1 μg of plasmid DNA and processed as described (15). Extraction prior to fixation was performed in 0.5% Triton X-100, 50 mM MES, 5 mM MgCl₂, and 3 mM EGTA (16). Jurkat cells were seeded (4 × 10³) on poly-L-lysine-coated coverslips, centrifuged for 1 min at 47 × g, fixed for 30 min at room temperature with 4% paraformaldehyde, and permeabilized with 0.05% saponin. Anti-V5 and anti-α-tubulin mAbs were used at 4 μg/ml, anti-β-tubulin mAb at 1 μg/ml, and anti-acetylated tubulin at 5 μg/ml. mAbs were revealed with AlexaFluor-coupled IgG1 secondary Abs (Molecular Probes) at 2 μg/ml. J1609–α-tubulin antisera were used at 1:500 and 1:100 dilutions, respectively. Affinity purified J1269 Abs were used at 5 μg/ml. Rabbit primary Abs were revealed with Alexa488-coupled secondary Abs (Molecular Probes) at 4 μg/ml. Confocal microscopy analyses (Figs. 5A and 6, A and B) were performed with a Zeiss LSM-510 microscope. Z-series of optical sections were performed at 0.5 μm increments. Images were acquired with settings allowing the maximum signal detection below the saturation limits. A middle optical section is shown. For double staining, sequential acquisitions were performed to avoid fluorescence contamination between the two channels. For the other figures, visualization was performed using a confocal laser scanning microscope (40× oil immersion lens).

**RESULTS**

Cloning of Jamip1, a Member of a Novel Family—As a step further in the study of Jak proteins, we conducted a yeast two-hybrid screen to identify proteins interacting with the FERM homology domain of Tyk2. The bait comprised the entire FERM domain of Tyk2, and the cDNA library was from Jurkat T cells. One positive clone encoded a partial protein of 261 aa flanked by a 3′-untranslated region of 213 nucleotides. From data base searches, several matching human and murine expressed sequence tags were found, all originating from neural tissue. The entire coding sequence was cloned by reverse PCR using adult human brain cDNA as the template (see “Experimental Procedures”). The sequence encodes a protein of 626 aa with a predicted mass of 73.1 kDa that was designated Jamip1 (see below). Recently, Couve et al. reported the identification by yeast two-hybrid screening of a neuronal specific protein that can associate with the R1 subunit of the GABAR receptor (17). This novel protein, named Marlin-1, is identical to Jamip1 (see “Discussion”).

**BLAST searches with the Jamip1 coding sequence predicted the existence of three highly related human genes with a conserved intron-exon organization (data not shown). Orthologues of the three Jamip genes were found in the murine genome. The chromosomal location and the available mRNA accession number of the human and the murine genes are shown in Table 1. An alignment of the three predicted human proteins is shown in Fig. 1. Note that, at the present time, limited data base information is available for Jamip3, and its amino acid sequence was therefore derived by comparative analysis of the genomic sequence with the coding sequences of Jamip1 and Jamip2. Jamip1 shares 58% aa identity with Jamip2 and the predicted Jamip3. The aa identity between the predicted hu...
man and murine proteins ranges from 66% for Jamip3 to 97% for Jamip1. Three Jamip-related genes were found in fish, but none were found in Drosophila and Caenorhabditis elegans.

Profile of Jamip1 and Jamip2 Expression—The mRNA expression profile of Jamip1 was analyzed in panels of human and murine tissues. Using 3′/H11032-specific Jamip1 probes, a single transcript of 2.5 kb was found highly expressed in adult brain and testis. Weaker signals were detected in other tissues (spleen, peripheral blood lymphocytes, lung, and intestine) (Fig. 2, A and B). Jamip2 expression in human tissues was also analyzed. A major transcript of 4 kb was highly expressed in brain, moderately expressed in thymus, spleen and lung, and weakly expressed in kidney, liver, and peripheral blood lymphocytes (Fig. 2A).

Rabbit polyclonal antisera were raised against two Jamip1-specific peptides (Fig. 1). Immunoprecipitation/Western blot analyses using either antisera revealed a single 73-kDa band in Jurkat cells, T cell blasts, Daudi (B lymphoblasts), and NKL, a natural killer cell line. No protein was detected in HT-1080 fibrosarcoma, THP1 monocytic, or Madin-Darby canine kidney epithelial cells. The antisera revealed two specific bands in rat pheochromocytoma PC12 cells and mouse brain extract (Fig. 2D). Thus, Jamip1 appears predominantly expressed in lymphoid cells in addition to neural tissues.

Jamip1 Can Homodimerize—Jamip proteins lack known conserved domains but are extremely rich in predicted α-helical coiled coils of varying lengths that are interrupted by non-helical regions ranging from 38 to 140 residues (Figs. 1 and 3A). Jamip1 contains two leucine zipper motifs, one of which is conserved in the family. Because coiled coil regions are known to serve as dimerization domains, we analyzed the ability of Jamip1, differentially tagged, to homodimerize in co-immunoprecipitation assays. FLAG-tagged Jamip1 was found to interact with V5-tagged Jamip1 but not with the control β-galactosidase protein (Fig. 3B, lanes 1 and 4). Truncated forms of Jamip1, comprising either the N-terminal or C-terminal portion (Fig. 3A), were generated and tested in this assay. Both forms were found to associate with full-length Jamip1 (Fig. 3B, lanes 2 and 3). We also analyzed the ability of C-terminal to interact with itself or with N-terminal. As shown in Fig. 3C, the C-terminal could self-associate but was unable to interact with the N-terminal region. Altogether, these data demonstrate that Jamip1 can homodimerize via the N-terminal and the C-terminal regions.

Interaction between Jamip1 and Jak Proteins—To confirm the interaction between Jamip1 and Tyk2 in a context other than that of yeast, we performed co-immunoprecipitation of the two endogenous proteins from Jurkat T cells. As seen in Fig. 4A, endogenous Tyk2 (134 kDa) was brought down with the anti-Jamip1 serum. Moreover, in 293T cells endogenous Tyk2 was shown to associate with transfected Jamip1 and C-terminal but not with N-terminal (Fig. 4B). These results demonstrated that both endogenous and ectopically expressed Jamip1 could associate with endogenous Tyk2 via the C-terminal portion.

Next, we asked whether Jamip1 could interact with the other
three Jak proteins expressed in Jurkat cells. Experiments performed with a panel of rabbit anti-Jak2 and Jak3 antisera did not yield conclusive results. On the other hand, endogenous Jak1, detected with a monoclonal Ab, was shown to co-immunoprecipitate with Jamip1 (Fig. 4C). The ability of Jamip1 to interact specifically with the Jak1 FERM domain was also monitored. For this monitoring, the Jak1 FERM was expressed as a GST fusion protein and incubated with in vitro translated Jamip1 or control β-galactosidase. As shown in Fig. 4D, the fusion protein retained Jamip1 but not β-galactosidase. Thus, Jamip1 can interact with the FERM domains of both Tyk2 and Jak1.

Given the well described involvement of Tyk2 and Jak1 in IFN-α/β signaling (18), we investigated whether the forced expression of Jamip1 constructs in 293T cells interfered with IFN-α/β signaling. Cells were co-transfected with an IFN-inducible luciferase reporter and Jamip1, N-ter, or C-ter. At high IFN doses, a 50% decrease in luciferase induction was consistently observed in cells transfected with Jamip1 or C-ter (Fig. 4D). Thus, overexpression of Jamip1 perturbed the transcriptional response to high IFN doses, i.e. upon high receptor occupancy. This finding provided additional evidence of the capacity of ectopically expressed Jamip1 or C-ter to interact with endogenous Jak proteins.

Jamip1 Associates with the Microtubule Cytoskeleton via the N-terminal Region—The subcellular localization of endogenous Jamip1 was studied in Jurkat T cells by confocal microscopy using affinity-purified anti-peptide polyclonal Abs. Staining was observed in organized filamentous structures that resembled the microtubule network as well as in non-filibrinous structures enriched in the cortex areas. To monitor a possible association with microtubules, we co-stained Jamip1 and tubulin. A clear overlap was observed, with Jamip1 heavily decorating microtubules. As shown in Fig. 4D, the fusion protein retained Jamip1 but not β-galactosidase. Thus, Jamip1 can interact with the FERM domains of both Tyk2 and Jak1.

To define which portion of Jamip1 directs its localization, we analyzed cells transfected with N-ter or C-ter. On cells permeabilized prior to fixation, N-ter was observed in association with stained microtubules (Fig. 5A, sections a–c). Next, we analyzed HT-1080 cells transiently transfected with Jamip1. A mild Triton X-100 extraction was performed prior to fixation to remove cytosolic proteins and preserve membrane-cytoskeletal associations. Co-localization of Jamip1 with tubulin was evident using either anti-Jamip1 Abs (Fig. 5A, sections d–f) or anti-V5 mAb (data not shown). Both Jamip1 and tubulin filamentous staining patterns were disrupted by the addition of the microtubule-depolymerizing agent nocodazole (see Fig. 6B, sections a and b), demonstrating that Jamip1 and polymerized microtubules are closely associated. It was noteworthy that, whereas tubulin staining disappeared, Jamip1 collapsed in a diffuse, non-filamentous pattern. Thus, the solubilization property of the protein in Triton X-100 did not depend solely on the presence of assembled microtubules.

To determine which portion of Jamip1 directs its localization, we analyzed cells transfected with N-ter or C-ter. On cells permeabilized prior to fixation, N-ter was observed in association with stained intermediate microtubules (Fig. 5B, sections a and b), and C-ter appeared predominantly nuclear (data not shown). In non-permeabilized cells the N-ter staining was unchanged, but C-ter appeared to be diffuse in the cytoplasm and the nucleus in a non-filamentous pattern (Fig. 5B, sections c...
and d). These results demonstrate that Jamip1 associates with the microtubule network via its N-terminal region.

**Jamip1 Influences Microtubule Dynamics**—In the course of these studies we noticed that, in cells expressing a high level of N-ter, microtubules assumed a wavy appearance with a high density of closely spaced and looped bundles (Fig. 6A). To further analyze the effects of Jamip1 and N-ter on microtubule organization, transiently transfected cells were challenged with nocodazole. A gentle nocodazole treatment (1 μM for 10 min) was sufficient to induce a complete microtubule depolymerization in the majority of Jamip1-expressing cells, whereas filamentous structures, resulting from incomplete depolymerization, were visible in untransfected cells (Fig. 6B, sections a and b). This result was indicative of the enhanced nocodazole sensitivity of Jamip1-expressing cells. Conversely, in N-ter-expressing cells doubly stained circular microtubule bundles were preserved even upon a stronger nocodazole treatment (10 μM for 60 min) (Fig. 6B, sections c and d). To substantiate this result, we compared the nocodazole sensitivity of a stable N-ter clone and control cells. Lengthy residual tubulin polymers were consistently more abundant in N-ter-expressing cells than in control HT-1080 parental cells (Fig. 6C). Because modification of tubulin subunits by acetylation marks older and more stable microtubules (19), we studied the effect of N-ter on microtubule stability by monitoring levels of acetylated tubulin. In N-ter-expressing cells, a strong acetylated tubulin staining was evident as compared with the weak or undetectable staining of untransfected cells (Fig. 6D).

The interaction between Jamip1 and microtubules was further investigated by biochemical fractionation and analysis of the distribution of tubulin and Jamip1. We used a gentle extraction protocol that removes soluble proteins to leave intact the cytoskeleton framework and the associated proteins (20). In a stable clone, Jamip1 resided in the detergent-insoluble fraction, whereas tubulin was equally distributed between soluble and insoluble fractions (Fig. 7A, lanes 1 and 2). Treatment of cells with nocodazole (10 μM, 4 h) resulted in a nearly complete shift of tubulin from the insoluble to the soluble fraction, whereas only a minor pool of Jamip1 was solubilized (Fig. 7A, lanes 3 and 4). Endogenous Jamip1 behaved similarly, as assessed by the analysis of Jurkat cells (Fig. 7B). Thus, the distribution of Jamip1 only partly matched the distribution of tubulin, and its insolubility could only in part be attributed to its association with microtubules.

Despite the drastic effect exerted by N-ter on microtubules (Fig. 6), when fractionation experiments were performed with
cells transiently expressing N-ter, no changes in the distribution of tubulin could be observed with respect to control glycoprotein transfected cells (Fig. 7C, upper sections). In view of the possibility that the fraction of N-ter-stabilized microtubules was too small to lead to a detectable shift in the distribution of total tubulin, we monitored the distribution of acetylated tubulin (Fig. 7C, lower sections). The amount of acetylated tubulin in the soluble fractions was comparable in the two transfected populations. However, the amount of acetylated, polymerized tubulin in the insoluble fractions was consistently higher in N-ter-transfected cells, and this finding was more evident upon nocodazole treatment (Fig. 7C, lower sections, compare lanes 3 and 4 with lanes 9 and 10). These results show that the N-terminal region is able to induce unique changes in microtubule organization, enhancing the pool of acetylated tubulin and reducing the sensitivity of microtubules to a depolymerizing agent. Both of these effects are indicative of an increased microtubule stability.

It is well known that the activity of various microtubule-associated proteins and their affinity to microtubules is modulated by Ser/Thr phosphorylation (21, 22). Thus, we analyzed the potential phosphorylation of Jamip1 on Ser/Thr residues in HT-1080-derived clones expressing Jamip1, N-ter, or C-ter. Upon in vivo treatment of cells with calyculin A, an inhibitor of Ser/Thr phosphatases, an evident shift in the migration of Jamip1 and C-ter, but not of N-ter, was observed, which is suggestive of multiple phosphorylation (Fig. 8A). A similar shift was evident upon analysis of endogenous Jamip1 in calyculin-treated Jurkat cells (Fig. 8B). Thus, Jamip1 can be post-translationally modified by Ser/Thr phosphorylation on its C-terminal portion.

**DISCUSSION**

We have identified Jamip1 as a protein that can associate with the FERM domain of Tyk2 and Jak1 and that localizes on microtubule polymers. In preliminary studies, we found that ectopically expressed Jamip2 shares similar properties. The restricted tissue expression profile of both genes and the scarce
**Fig. 5. Subcellular localization of Jamip1.** A, Jurkat cells were fixed, permeabilized, and double-stained with anti-Jamip1 Abs (affinity purified anti-J1269-286) (a) and anti-β-tubulin mAb (b). The image in section c is the merged image of those in sections a and b. HT-1080 cells transfected with Jamip1 were permeabilized (see “Experimental Procedures”), fixed, and double-stained with anti-Jamip1 Abs (d) and anti-α-tubulin mAb (e). Images were analyzed by confocal microscopy (see “Experimental Procedures”). Both endogenous (a and b) and transfected (c and d) Jamip1 co-localize with tubulin. B, HT-1080 cells were transfected with N-ter (a and b) or C-ter (c and d). Cells were permeabilized and fixed (a and b) or directly fixed (c and d) and double stained with anti-J1269-286 (a) or anti-J1609-626 (b) Abs and anti-α-tubulin mAb (b and d). The N-ter protein stained microtubules, whereas C-ter was diffuse throughout the cell in a non-filamentous pattern.

**Fig. 6. Effect of Jamip1 and N-ter on microtubule organization.** A, HT-1080 cells transfected with N-ter were permeabilized, fixed, and double-stained with anti-Jamip1 Abs (a) and anti-α-tubulin mAb (b) and analyzed by confocal microscopy. The image in section c is the merged image of those in sections a and b. Highly expressed N-ter co-localizes with bundled microtubules. B, HT-1080 cells were transfected with Jamip1 and treated with 1 μM nocodazole (noco) for 10 min (a and b) or transfected with N-ter and treated with 10 μM nocodazole for 60 min (c and d). Cells were double-stained as in panel A and analyzed by confocal microscopy. Cells in each field were visualized by Nomarski imaging, and their edges were outlined. Note the complete depolymerization of microtubules only in Jamip1-positive cells. Conversely, N-ter stabilized microtubules against the effect of nocodazole. C, control HT-1080 cells (a–c) and a stable N-ter clone (d–f) were left untreated (a and d) or treated with 3 μM nocodazole for 30 min (b and e) or 10 μM nocodazole for 60 min (c and f). Cells were processed as in panel A and stained with anti-α-tubulin mAb. Residual tubulin polymers appear longer in drug-treated N-ter clone (cl. N) cells than in treated HT-1080 cells. D, HT-1080 cells transfected with N-ter were double stained with anti-Jamip1 Abs (a) and anti-acetylated (acetyl.) tubulin mAb (b). Cells in each field were visualized by Nomarski imaging, and their edges were outlined. The N-ter positive cell shows a stronger acetylated tubulin staining than the surrounding untransfected cells.
Fig. 7. Biochemical fractionation. A, HT-1080 cells stably expressing Jamip1 were treated with 10 μM nocodazole (noco) or 10 μM Taxol for 4 h and then subjected to selective detergent extraction (see “Experimental Procedures”). 10 μg of soluble proteins (sol) and an equivalent volume of insoluble material (ins) were resolved by SDS-PAGE, transferred, and immunoblotted using anti-α-tubulin mAb (upper section of panel) or anti-V5 mAb (lower section of panel). B, Jurkat cells, treated with 40 μM nocodazole for 4 h or left untreated, were subject to detergent extraction (see “Experimental Procedures”). 10 μg (upper section of panel) and 40 μg (lower section of panel) of soluble proteins and equivalent volumes of insoluble material (ins) were blotted with anti-α-tubulin (upper section of panel) and anti-Jamip1 Abs (lower section of panel). C, HT-1080 cells were transiently transfected with β-galactosidase or N-ter (N) and treated for 4 h with two doses of nocodazole. Cells were subject to detergent extraction and analyzed as in panel A. Immunoblotting was with anti-α-tubulin (upper section of panel) or anti-acetylated tubulin mAbs (lower section of panel).

Fig. 8. Phosphorylation of Jamip1 on Ser/Thr residues. A, stable HT-1080-derived clones (cl.) expressing Jamip1 (J1), N-ter (N), or C-ter (C) were treated for 20 min with or without 1 μM calyculin A (cal.). Proteins were analyzed by immunoblotting (WB) with anti-V5 mAb. B, Jurkat cells were treated as in panel A, and 40 μg of total lysate was immunoblotted using anti-Jamip1 Abs. Note the reduced mobility of Jamip1 and C-ter in calyculin-treated cells.

The number of existing Jamip3 expressed sequence tags, all of neuronal origin, strongly argue for a specialized function of this novel family. The main feature of Jamip proteins is the presence of several non-contiguous α-helical regions with high potential to form coiled coils (23). Accordingly, we found that Jamip1 can self-associate in vivo via homophilic interactions involving the N-terminal or C-terminal regions.

In Jurkat T cells, endogenous Jamip1 appears in filamentous structures that overlap with microtubules and concentrate around the centrosomal region. Small punctuate structures of unknown nature are occasionally visible. When ectopically expressed in fibroblasts, Jamip1 is largely localized along the entire length of the microtubules, including the centrosomal region, in both interphase and mitotic cells. The endogenous Jak proteins are not abundant and are below immunofluorescence detection limits. We reported previously that transfected Tyk2 is found to be diffuse throughout the cell and enriched at the plasma membrane (15). Despite the biochemical evidence of Jamip1/Tyk2 interaction, we failed to detect their co-localization because, presumably, the fraction of Tyk2 associated with Jamip1 is rather small.

A hallmark of some microtubule-associated proteins is internal repeats with high pI that mediate electrostatic interactions with negatively charged tubulin (24). In Jamip1, the N-terminal region mediates the association with microtubules. N-ter lacks overt, repeated sequences or homology with known microtubule binding domains, but, as opposed to the full-length protein or C-ter, its calculated pI is 9.12. When highly expressed, N-ter drastically affects the organization of microtubules that appear to be bundled, stabilized against the depolymerizing effect of nocodazole, and enriched in acetylated tubulin. It is conceivable that, when deleted from the C terminus, the protein acquires higher stability. Above a threshold level, dimers or oligomers could have microtubule bridging activity leading to bundle formation. In the context of the native protein, C-ter may restrain this process by maintaining the protein in a “physiological” conformation and/or by targeting N-ter to privileged partners.

Despite the co-localization of tubulin and Jamip1, upon selective extraction a different biochemical distribution of the two proteins was observed, because Jamip1 was found almost exclusively in the detergent-insoluble fraction. Moreover, the nearly complete solubilization of tubulin in nocodazole-treated cells was accompanied by the solubilization of only a minor pool of Jamip1. These results rule out an association of Jamip1 (or N-ter) with tubulin heterodimers, as is the case for some mi-
bule network is not required for STAT-mediated expression of cytoskeletal structures (18, 33). The integrity of the microtubule binding repeats, known targets of proline-directed kinases. Two of the five proline residues present in the C-terminal region of Jamip1 are in a conserved motif (Thr-Pro-Ala-Thr-Pro) that may represent a target of proline-directed kinases 

Jamip1 contains potential Ser/Thr phosphorylation target residues in its C-terminal portion. Interestingly, the affinity of various microtubule-associated proteins to microtubules is controlled by multiple and sequential Ser/Thr phosphorylation (21, 22). In related microtubule-associated proteins, Ser/Thr-Pro motifs, located in a proline-rich region adjacent to the microtubule binding repeats, are known targets of proline-directed kinases. The five proline residues present in the C-terminal region of Jamip1 are in a conserved motif (Thr-Pro-Ala-Thr-Pro) that may represent a target of proline-directed kinases (Fig. 1).

Jak tyrosine kinases have been classically assigned to pathways initiated at the plasma membrane by helical cytokine receptors. Jak activation initiates a phosphorylation cascade leading to direct activation of signal transducers and activators of transcription (STAT) factors and gene transcription (3). Jak5 may also be necessary to initiate additional signaling cascades involving phosphatidylinositol 3-kinase, mitogen-activated protein kinase, or stress-activated kinases that ultimately impinge on gene transcription and cytoskeletal structures (18, 33). The integrity of the microtubule network is not required for STAT-mediated expression of IFN-α-inducible genes, at least in fibroblasts (34, 35). Alteration of tubulin polymerization and increased resistance of microtubule polymers to colchicine were found to be induced by a growth hormone (34). This effect, shown to be Jak-dependent, suggests that some of the activities of this cytokine may involve changes in the microtubule dynamic equilibrium.

We found that forced expression of Jamip1 or of its C-terminal portion in fibroblasts interfered with the IFN-α transcriptional response, most likely as a result of the sequestration of Jaks away from the IFN receptor. A critical question that remains open concerns the potential role of Jamip1 in cytokine-driven signaling. No evidence of IFN-α or IFN-γ-induced tyrosine phosphorylation of endogenous Jamip1 could be obtained in Jurkat cells even if, when overexpressed with Jak1, Jamip1 is found tyrosine phosphorylated in the C-terminal portion (data not shown). Given the restricted tissue expression of Jamip proteins, it is possible that Jak-mediated cytokine signaling may require a spatial or directional element provided by the Jamip microtubule scaffold only in response to specific cytokines and in certain cell types. Another possibility is that a regulated Jamip/Jak interaction may play a role in cytokine-independent dynamic events such as, for example, those related to directional transport. The finding that Jak2 is one of the enzymes regulating the activity of the p97/VCP protein is intriguing, since this protein plays an essential function in endoplasmic reticulum vesicle fusion and membrane transport processes (12, 36).

Marlin-1, alias Jamip1, was recently identified as an interacting partner of GABAR-R1, a subunit of the heterodimeric G protein-coupled GABA receptor (17). In this study, the expression of Marlin-1/Jamip1 in hematopoietic cells was not reported. Immunofluorescence staining of cultured hippocampal neurons or transfected COS cells revealed a granular distribution of the protein. Conversely, our results underline a prominent microtubule association of both endogenous and transfected Jamip1. We did, however, notice some positive staining in non-fibrous or punctuate structures in Jurkat cells. Interestingly, the study of Cousse et al. (17) points to a role for Marlin-1 in the regulation of the level of the GABA-R2 subunit via a direct binding to the corresponding mRNA. As stated by the authors, the RNA binding property of Marlin-1 supports its involvement in the neuronal transport of ribonuclear particles, a process known to require intact microtubules (37). The presence of Marlin-1/Jamip1 along microtubule tracks, as we describe here, is compatible with its being a component of the transport machinery.

The high degree of conservation of Marlin/Jamip proteins in vertebrates and their restricted expression profile argue for a common function in neuronal and lymphoid cells. Despite evident dissimilarities between circulating lymphoid cells and neurons, parallels have been made between the intercellular contact sites at the neuronal synapse and at the so-called immune synapse, i.e., the interface between effector T cells and antigen-presenting cells. In both systems, Marlin-1/Jamip1 could play a role in cytoskeletal rearrangements, maintenance of the synapse, transport of ribonuclear particles, or distribution of vesicles for the polarized release of soluble mediators. Although the evaluation of the physiological significance of the association of Jamip1 with at least two Jak family members, Tyk2 and Jak1, awaits more work, further study of this novel family may bring new insights into cytoskeletal mediated processes common to neurons and lymphocytes. A future challenge will be to investigate whether mutations in Marlin/Jamip genes are responsible for neuro-degenerative diseases and/or lymphocyte dysfunction.

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