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The human mitotic kinesin KIF18A binds protein phosphatase 1 (PP1) through a highly conserved docking motif

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ABSTRACT

Protein phosphatase 1 (PP1), a serine/threonine protein phosphatase, controls diverse key cellular events. PP1 catalytic subunits form complexes with a variety of interacting proteins that control its ability to dephosphorylate substrates. Here we show that the human mitotic kinesin-8, KIF18A, directly interacts with PP1γ through a conserved RVxF motif. Our phylogenetic analyses of the kinesins further uncovered the broad conservation of this interaction potential within the otherwise highly diverse motor-protein superfamily. This suggests an ancestral origin of PP1 recruitment to KIF18A and a strategic role in human mitotic cells.

1. Introduction

Serine/threonine protein phosphatase 1 (PP1) is a highly conserved eukaryotic enzyme and a key cellular regulator due to its dominant role in serine/threonine protein (de)phosphorylation. 98% of phosphorylation events that occur in the human proteome are Ser/Thr phosphorylations and PP1 together with PP2A, accounts for >90% of these dephosphorylation events [1–3]. Protein kinases and phosphatases, respectively, add or remove phosphate moieties, thereby influencing substrate function. Throughout evolution, protein kinases developed into a large enzyme family, each with a particular substrate recognition motif. Conversely, the Ser/Thr protein phosphatase numbers remained small, developing their substrate recognition via complex formation between a canonical, low specificity catalytic subunit and a large array of regulatory proteins. The best example is PP1, where each complex consists of a catalytic subunit (PP1) bound with a regulatory protein, mostly via short, degenerate interaction motifs (RVxF; SILK; MyPHONE) in the primary sequence of the latter [1,4].

Mammalian species encode for 3 ubiquitously expressed isoforms of PP1 (α, β, γ) which, in non-mitotic cells, localize to both overlapping and distinct subcellular compartments [5]. The overall number of known PP1 interacting proteins has grown exponentially in recent years [4,6], which is commensurate with newly discovered roles for protein phosphatases in the regulation of cell growth and division, signal transduction and gene expression. Pools of human PP1 isoforms relocate to specific subcellular positions both during mitosis and in response to external triggers [4]. The mechanisms underlying these relocations remain largely unexplored.

Organelles and molecules are transported through the eukaryotic cell via 3 motor protein classes (dyneins, kinesins and myosins) and an extensive cytoskeleton. Their functionality is carefully regulated. Mitotic kinesins are kept functionally inactive during interphase via nuclear sequestration [7]. This may, however, offer an opportunity to upload their cargo, ready for mitotic onset. We screened the nuclear PP1 interactome in proliferating HeLa cells for the presence of motor proteins and identified the human mitotic kinesin-8 KIF18A as a novel PP1 interactor with a functional interacting RVxF motif. Sequence analyses further showed that the RVxF motif within KIF18A motif is better conserved than all other RVxF motif within the kinesin superfamily.

Abbreviations: PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; MTs, microtubules.

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It also withstood evolutionary pressure, indicative of a key mitotic role for PP1 binding to this kinesin-8 member.

2. Materials and methods

Chemicals were obtained through VWR or Bioshop Canada, unless otherwise indicated.

2.1. Cell lines and plasmids

HeLa cells were obtained from ATCC. Stable cell lines (HeLa$^{GFP\text{-}PP1\alpha\text{-}\gamma}$; HeLa^{GFP}) were established and transient transfections performed as previously described [5]. Cells were arrested at the G2/M checkpoint with nocodazole (40 ng/ml for 16 h) or at G1/S via a double thymidine block (2.5 mM thymidine, 18 h; with 9 h intermediate release). KIF18A was subcloned from an Open Biosystems FL cDNA using unique restriction sites for KpnI and Bam H1 and inserted into an mCherry-C1 mammalian expression vector.

2.2. Protein extracts and immunoprecipitations

Nuclear protein extracts, microcystin-Sepharose based chromatography and peptide displacement were described in [6]. SDS–PAGE and Western blot analyses were performed according to standard protocols. Antibodies against specific proteins or control IgGs were covalently coupled to Protein A-Sepharose beads (Invitrogen) with dimethylpimelimidate (Sigma) according to the manufacturer's protocol. Antibodies were from Bethyl Laboratories (KIF18A), Santa Cruz (sc594 -cyclin B1, sc7482-pan PP1) and Roche (GFP mAb).

2.3. KIF18A-PP1 co-immunoprecipitation

Unsynchronized HeLa cells were harvested, proteins extracted (50 mM Tris pH 7.5, 1 mM EDTA/EGTA, 150 mM NaCl, 0.5% NP40, 0.5 mM DTT, 1 mM PMSF, 1 mM Benzamidine, 2 μg/ml Leupeptin, 1 μg/ml Pepstatin) and incubated with Protein A-Sepharose coupled KIF18A/IgG antibodies (2 h 4 °C end over end). Isolates were washed 3 times (PBS + 0.3 M NaCl – 50 Column Volumes, 5 min each) and beads boiled in sample buffer.

2.4. Quantitative comparison of GFP-PP1 complexes

For these experiments, HeLa^{GFP}, HeLa$^{GFP\text{-}PP1\alpha}$ and HeLa$^{GFP\text{-}PP1\gamma}$ stable cell lines were differentially labeled by growth in SILAC (Stable Isotope Labeling by Amino acids in Culture) DMEM supplemented with 10% dialyzed FBS and 100 U/mL penicillin/streptomycin and containing either "light" l-arginine and l-lysine (Sigma–Aldrich; R0K0), "medium" l-arginine ^{13}C and l-lysine 4,4,5,5-D4 (Cambridge Isotope Laboratories; R6K4) or "heavy" l-arginine $^{13}C^{15}N$ and l-lysine $^{13}C^{15}N$ (Cambridge Isotope Laboratories; R10K8). To compare mitotic complexes for GFP-PP1α vs. GFP-PP1γ (with GFP alone as the built-in negative control), cells were nocodazole-arrested, harvested by mitotic shake-off and proteins extracted by sonication in RIPA buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% deoxycholate, and Roche protease inhibitor cocktail). To compare mitotic vs. interphase complexes for PP1γ, HeLa$^{GFP\text{-}PP1\gamma}$ cells were labeled with R0K0 or R6K4, and the latter nocodazole-arrested.

Equivalent amounts of each lysate were incubated with GFP-TrapA (Chromotek) for 1 h (4 °C, end over end), washed and combined, and proteins eluted, separated by 1D SDS–PAGE, and trypsin-digested for MS analysis on an LTQ Orbitrap XL hybrid mass spectrometer as described previously [8]. MaxQuant software v1.2.7.4 [9] was used for database searching (against the human Uniprot database) and quantitation.

2.5. GFP-PP1/KIF18A co-immunoprecipitation

Unsynchronized or nocodazole-arrested HeLa^{GFP} and HeLa$^{GFP\text{-}PP1\gamma}$ cells were harvested and proteins extracted by sonication in RIPA buffer. Equivalent amounts of lysate were incubated with GFP-TrapA (Chromotek) for 1 h (4 °C, end over end), beads washed 3 times with RIPA buffer and boiled in sample buffer to elute proteins for further analysis.

2.6. Modification of the PP1 binding motif in KIF18A and protein purification

The C-terminal KIF18A gene (aa 555-898) was amplified from the human cDNA and cloned into the pET101 vector (Invitrogen). The putative PP1 binding $KK_{612}VVVW_{616}AD$ motif was mutated to non-binding $KK_{612}AVVA_{616}AD$ version (called KIF18A-RARA hereafter) by site directed mutagenesis (Stratagene). All constructs were sequence verified by DNA sequencing (University of Calgary). pET101-C terminal KIF18A WT or RARA mutant were transformed into BL21 (DE3) cells, grown in LB-Amp with 0.5% glucose (w/v) to $O.D_{600nm}$ of 1.0 and induced with 0.3 mM IPTG for 2 h at 37 °C. The cells were harvested and snap-frozen (−80 °C) until further use. Cell pellets were resuspended in lysis buffer (25 mM Tris pH 7.5, 150 mM NaCl, 0.5% NP-40, 1 mM PMSF and 0.5 mM Benzamidine) and lysed with a French press (Sim-Aminco; 2 runs at 83 MPa exit pressure) and debris pelleted by centrifugation (20,000 rpm at 4 °C for 45 min). The supernatant was loaded onto a pre-equilibrated Ni–NTA column (1.5 mL) and incubated end over end for 90 min at 4 °C. Column was washed with wash buffer (25 mM Tris pH 7.5, 1 M NaCl, 10 mM imidazole, 0.05% Triton X-100, 1 mM PMSF and 0.5 mM Benzamidine) before application of elution buffer (lysis buffer with 300 mM imidazole). The eluted proteins were concentrated using Amicon filters and frozen at −80 °C.

2.7. PP1γ – KIF18A in vitro binding assay

PP1γ was purified using microcystin-Sepharose beads as described previously [6]. Purified PP1γ (6 μg) was mixed with equal amounts (10 μg) of either wild type C-terminal fragment of KIF18A or RARA KIF18A in 500 μL binding buffer (25 mM Tris pH 7.5, 5% glycerol, 150 mM NaCl and 10 mM imidazole) and allowed to interact for 30 min at 4 °C. Ni–NTA beads were equilibrated with binding buffer and blocked with 1 mg/mL BSA solution to prevent non-specific binding. Blocked beads (40 μL) were added to each sample and incubated end over end for 2 h at 4 °C. Beads were washed 3 times with wash buffer (25 mM Tris pH 7.5, 5% glycerol, 1 M NaCl, 10 mM imidazole and 1% Tween-20) before eluting the proteins with 2X sample buffer. Eluates were run on 12% SDS–PAGE gels, transferred onto nitrocellulose membrane and probed with either PP1 antibody (Santa Cruz, Sc7482) or His antibody (Qiagen).

2.8. HeLa cell microscopy

HeLa$^{GFP\text{-}PP1\gamma}$ cells were cultured in glass-bottomed dishes (WILLCO; Intracel) for transient transfection with the mCherry-KIF18A plasmid (Effectene, QIAGEN). Images were acquired using a DeltaVision CORE widefield fluorescence system fitted with a CoolSNAP coupled-charge device (CCD) camera (Roper Scientific) and Weatherstation environmental chamber to maintain 37 °C. Before imaging, growth medium was replaced with Phenol Red-free CO_2 independent medium (Invitrogen). DNA was stained by incubating cells (20 min) in medium with 0.25 μg/ml Hoechst

No. 33342 (Sigma–Aldrich). Cells were imaged using a 60× NA 1.4 Plan-Apochromat objective (Olympus), with 20 × 0.5 μM optical sections collected using the appropriate filter sets (Chroma Technology Corp.). The microscope was controlled and images processed by SoftWorX acquisition and deconvolution software (GE Healthcare).

2.9. Phylogenetic analyses

Human KIF18A was used as query to probe various online databases for related sequences via pBLAST and tBLASTn approaches. Sequences were aligned with CLUSTALX and Genedoc, duplicates removed and a bootstrapped (1000 re-samplings) neighbor-joining tree was generated with full length sequences according to standard parameters. PP1-binding (RVxF) and coiled-coil motifs were identified based on http://elm.eu.org (see Supplementary data for complete tree). The resulting tree was condensed to indicate evolutionary bifurcations and coinciding motif presence.

3. Results and discussion

3.1. The kinesin-8 motor protein KIF18A is a novel PP1 interactor

Previously, we purified PPP complexes by incubating a nuclear HeLa cell extract with a microcystin (MC)-Sepharose matrix and eluting PP1 binding partners by peptide displacement chromatography [6]. Most PP1 binding partners contain a short canonical motif (“RVxF”) in their primary sequence, essential for binding to PP1. We used an excess of a highly matching peptide (“RVRW”) to release PP1 interactors from matrix-bound PP1. False positives were eluted with a control peptide (“RARA”), prior to RVRW elution, while remaining partners were eluted with 3 M isothiocyanate (SCN). Eluted proteins were separated via SDS–PAGE, visualized and analyzed by mass spectrometry, as described in [6]. Here we concentrated on the motor proteins present, in particular dyneins and kinesins, because the latter is the largest motor protein class and ample research exists on the interaction between PP1, MYPT and myosin [10].

Within the nuclear PP1 interactome, we identified cytosolic dynein heavy chain 1 and 2 (data not shown) and 2 kinesins (Table S1). The dynein chains possess an RVxF-motif, yet their location on the SDS–PAGE gel was significantly below their estimated size, suggesting these were degradation products. Other identified peptides were from a kinesin-4 (KIF21A) and a kinesin-8 (KIF18A) subfamily member with 7 and 4 peptide matches, respectively (Table S1). The predicted mass of identified KIF18A was consistent with the full length protein. Contrary to KIF21A, KIF18A contains a putative PP1 binding RVxF-motif ($KK_{612}VVVW_{616}AD$) and is localized in the nuclei of proliferating cells [5,7]. Furthermore, kinesin-8 homologs in *Schizosaccharomyces pombe* (Klp5/6) were recently identified as PP1 binding proteins with a role in spindle checkpoint silencing [11].

We corroborated this MS-based identification via Western blot analysis of the peptide displacement elutions (Fig. 1A). KIF18A is released from the MC-Sepharose matrix by the PP1-binding peptide (RVRW), but not by prior elution with the control peptide (RARA). Moreover, elution with the RVRW peptide is necessary and sufficient, because there is no detectable KIF18A signal in the subsequent thiocyanate (SCN) eluate. These results strongly suggest that human PP1 interacts with the kinesin-8 KIF18A via its putative RVxF motif. These results were further supported by co-precipitation of endogenous PP1 with endogenous KIF18A from asynchronous HeLa cells (Fig. 1B). *In-vitro* binding assays with His-tagged C-terminal fragment show a direct interaction of KIF18A with PP1 (Fig. 1C). We also show that the interaction of KIF18A with PP1 is via the $KK_{612}VVVW_{616}AD$ motif as the mutated version of KIF18A (RARA) is not able to bind PP1 (Fig. 1C).

3.2. KIF18A remains a member of the PP1 interactome at mitotic onset

The low protein level of KIF18A in asynchronous, proliferating cells negatively influences mass spectrometry-based identification and immunoprecipitation alike. Indeed, we (Fig. S1) and others [12] observed that KIF18A protein levels are cell cycle dependent, with levels peaking in G2 and mitosis. We therefore studied the interaction between PP1 and KIF18A in unsynchronized HeLa cells and at mitotic onset. We focused on PP1γ because proteomic analysis of GFP-PP1α and GFP-PP1γ immunoprecipitated from G2/M-arrested HeLa cells identified KIF18A as a PP1γ-specific interactor (Fig. 1D). To validate this interaction, proteins were extracted from either asynchronous or G2/M arrested $HeLa^{GFP\text{-}PP1\gamma}$ and $HeLa^{GFP}$ cell lines, and GFP or GFP-PP1γ immunodepleted using the high affinity GFP-TrapA reagent. Lysates and enriched proteins were separated via SDS–PAGE and probed for GFP or GFP-PP1γ and endogenous KIF18A. Fig. 1E shows that KIF18A is significantly enriched by GFP-PP1γ compared to GFP alone in both cell cycle stages. In addition, a quantitative mass spectrometry approach revealed KIF18A as the protein with greatest increased PP1 association during mitosis (Fig. S2). Hence, KIF18A readily interacts with PP1, particularly at mitotic onset when KIF18A levels are increasing (Fig. S1), most likely in preparation for its role in chromosome congression. Kinesin-8 subfamily members have long been implicated in mitotic regulation, yet the potential for PP1-binding remained unnoticed. Recently, however, the *S. pombe* kinesin-8 members (Klp5, 6) were identified as crucial players for silencing the spindle assembly checkpoint at the metaphase/anaphase transition. This required binding of PP1 by Klp5/6, more so than their microtubule depolymerisation capacity [11]. We have now identified a similar interaction between KIF18A and PP1 in metazoan cells, suggesting that PP1 binding may be of equal importance for the role of this and related kinesin-8 family members in chromosome congression. This observation is now supported by another recent work [13].

3.3. The potential for PP1 interaction within the kinesin-8 family members

A recent study to define the last common eukaryotic ancestor of the kinesin superfamily showed its complex origin with multiple gene duplications and secondary losses during evolution [14]. If PP1 binding is indeed a key element for kinesin-8 members, we expect the RVxF motif to be conserved within the subfamily. We therefore performed a directed phylogenetic study. Human KIF18A and *S. pombe* Klp5/6, kinesin-8 members with PP1 binding capacity, both consist of an N-terminal motor domain, coiled-coil motifs and a PP1-binding motif in the C-terminal end. To perform phylogenetic analyses on the PP1-binding motif within kinesin-8 members, we collected all available sequences using full-length human KIF18A as query, contrary to [14] in which mere motor domains from 45 representative eukaryotic genomes were used.

Protein sequences were aligned and used to produce a rooted, bootstrapped neighbor-joining tree (Fig. S3). The human KIF2A sequence belongs to the closest related (kinesin-13) subfamily [14] and served as outgroup. Similar to Wickstead and colleagues [14], we found eukaryotic genomes contain up to 3 groups within the kinesin-8 subfamily, namely KIF18A (8A), KIF18B (8B) and KIF19. To better highlight the evolutionary relationships between kinesin-8 subfamily members and their respective motif organizations, we condensed the complete tree of the kinesin-8 subfamily (Fig. S3) to the most significant members and/or the encompassing classification name. We indicated the position of the motor

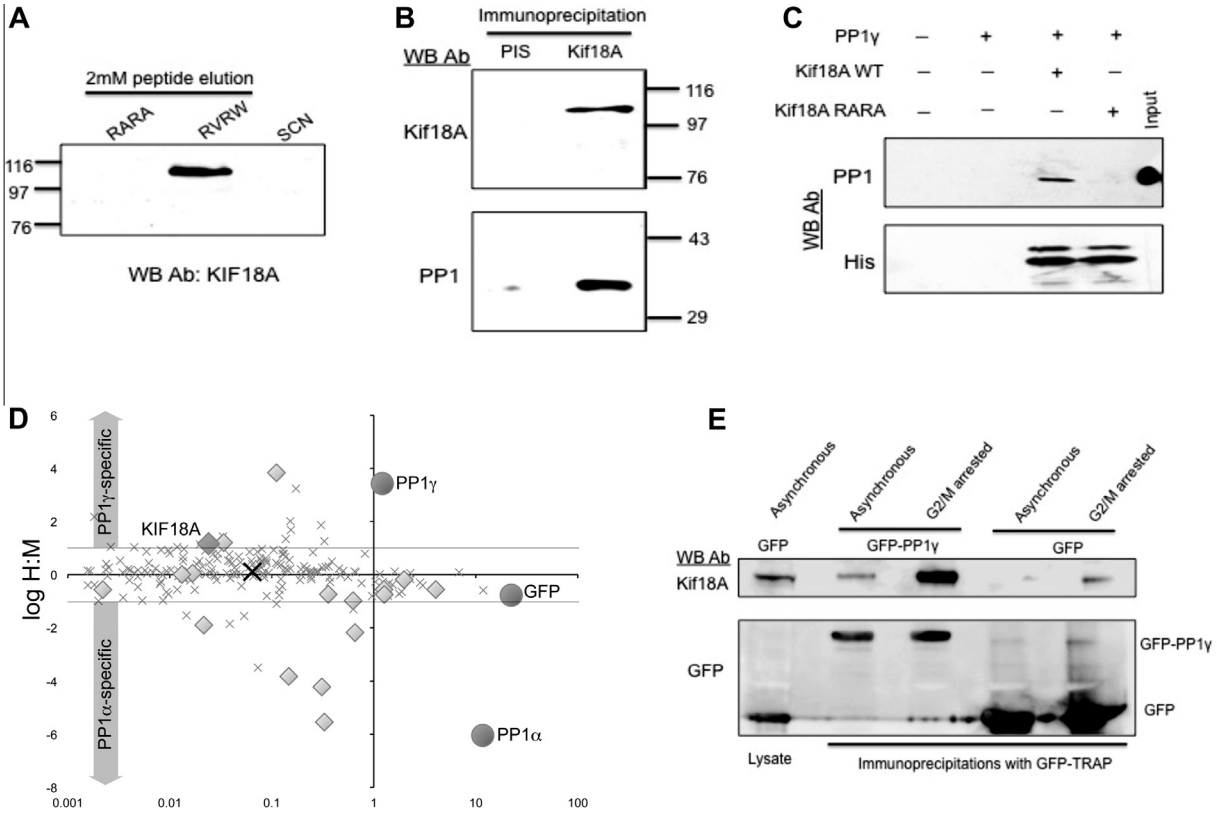

Fig. 1. The human kinesin-8 KIF18A interacts with PP1. (A) Protein phosphatase complexes were enriched from the nuclear proteome of unsynchronized HeLa cells with a microcystin (MC)-Sepharose matrix. PP1 interactors were eluted via sequential peptide displacement chromatography with a control peptide (''RARA''), a high affinity peptide (''RVRW'') and 3 M iso-thiocyanate (SCN). Eluates were separated via SDS–PAGE and verified by Western blot analyses for the presence of KIF18A. (B) HeLa extracts were incubated with either α-KIF18A or IgG antibodies, each cross-linked to Protein A-Sepharose beads. Enriched proteins were washed, eluted and separated by SDS–PAGE prior to Western blot analysis for the presence of KIF18A and PP1. (C) Bacterially expressed and purified PP1γ and His6-Cterminal KIF18A WT or RARA mutant were incubated together and mixed with BSA-blocked Ni–NTA beads. After allowing them to bind for 2 h at 4 °C, the bound proteins were eluted with 2X SDS–PAGE cocktail and analyzed for presence of PP1. (D) HeLa^{GFP}, HeLa$^{GFP\text{-}PP1\alpha}$ and HeLa$^{GFP\text{-}PP1\gamma}$ cells were labeled with light (L), medium (M) and heavy (H) Arg and Lys, respectively, prior to affinity purification and mass spectrometric analysis. Plotting the log H:M ratios calculated for each identified protein vs. their relative abundance (summed peptide intensity, normalized by molecular weight) highlights targeting subunits (diamonds) that are equally enriched with both PP1 isoforms (log H:M ~ 1) and those that are enriched more with PP1γ (ratio > 1 log above median) or PP1α (ratio > 1 log below median). KIF18A (gray diamond) is indicated, as is the median value (black X). (E) KIF18A and PP1 interact in cells arrested at mitotic onset. HeLa^{GFP} and HeLa$^{GFP\text{-}PP1\gamma}$ cells were either kept untreated (proliferating) or arrested at mitotic onset (G2/M). Cells were lysed and incubated with the GFP-TrapA reagent. Enriched proteins from each of the 4 fractions were separated by SDS–PAGE and probed for the presence of GFP or GFP-PP1γ (lower panel) and KIF18A (upper panel). A representative lysate is shown for the HeLa^{GFP} cell line.

domains and the number of coiled-coil and PP1-binding motifs (Fig. 2).

In line with the nature of kinesin evolution, the KIF18A and KIF18B groups are most likely due to a recent gene duplication event. Most interesting is the prevalence of PP1-binding motifs in KIF18A members, which are completely lacking in KIF18B members. The KIF19 sequences may also have risen from a duplication event with the ancestral KIF18. Interestingly, potential PP1 binding motifs are scattered throughout the KIF19 group, and motif sequences differ from the highly conserved KIF18A motif. Each group has early eukaryote members, which makes it difficult to define whether the ancestral KIF18 or KIF19 is more closely related to the founding kinesin-8 member. The presence of a PP1-binding motif in Brown algae, Fungi, unicellular Holozoans and regressed Animalia up to Metazoans, however, strongly suggests that this motif was part of the ancestral kinesin-8, supporting the idea that PP1 association is fundamental to KIF18A function. Secondary losses, again reminiscent of overall kinesin evolution [14], may have resulted in the docking motif's absence from KIF18B and some KIF19 members.

The kinesin superfamily has grown exponentially, most likely reflecting the increasing need for specialized motor proteins in multi-cellular organisms. We analyzed the distribution of PP1-binding motifs within the kinesins in select species. *S. pombe* has 9 identified kinesins yet only the kinesin-8 members (Klp5/6) contain an RVxF motif. For *Saccharomyces cerevisiae*, we identified RVxF motifs in 2 of the 6 kinesins, namely kinesin-5 (Cin8) and kinesin-8 (Kip3) members. Humans currently list 45 kinesins, 18 of which (subfamily 1, 3–5, 7–9, 11, 13) possess putative RVxF motifs (Fig. S4). Some motifs are functionally significant in particular organisms [15] yet none of them is as broadly conserved as the KIF18A RVxF motif.

Within the fungal sequences we observed one kinesin-8 member (Fig. S3) in the Mucormycotina and Basidiomycota, although only the latter possessed an RVxF motif, based on currently available sequences. Within the Ascomycotina, the Schizosaccharomycetes (*S. pombe* and japonicas) each have 2 kinesin-8 members, one of which has 2 RVxF motifs; the Saccharomycotina each have 1 member with 1 motif, but the last subphylum, the Pezizomycotina (filamentous Ascomycetes), display 1 member per species and each with a tandem PP1-binding motif, similar to Schizosaccharomycetes. This suggests that the presence of a PP1 binding motif is part of the ancestral fungal kinesin-8 and may have suffered from the occasional secondary loss. Simultaneously, a duplication may have led to the tandem motif we observe in the Schizosaccharomycetes and Pezizomycotina. We also observed sequence

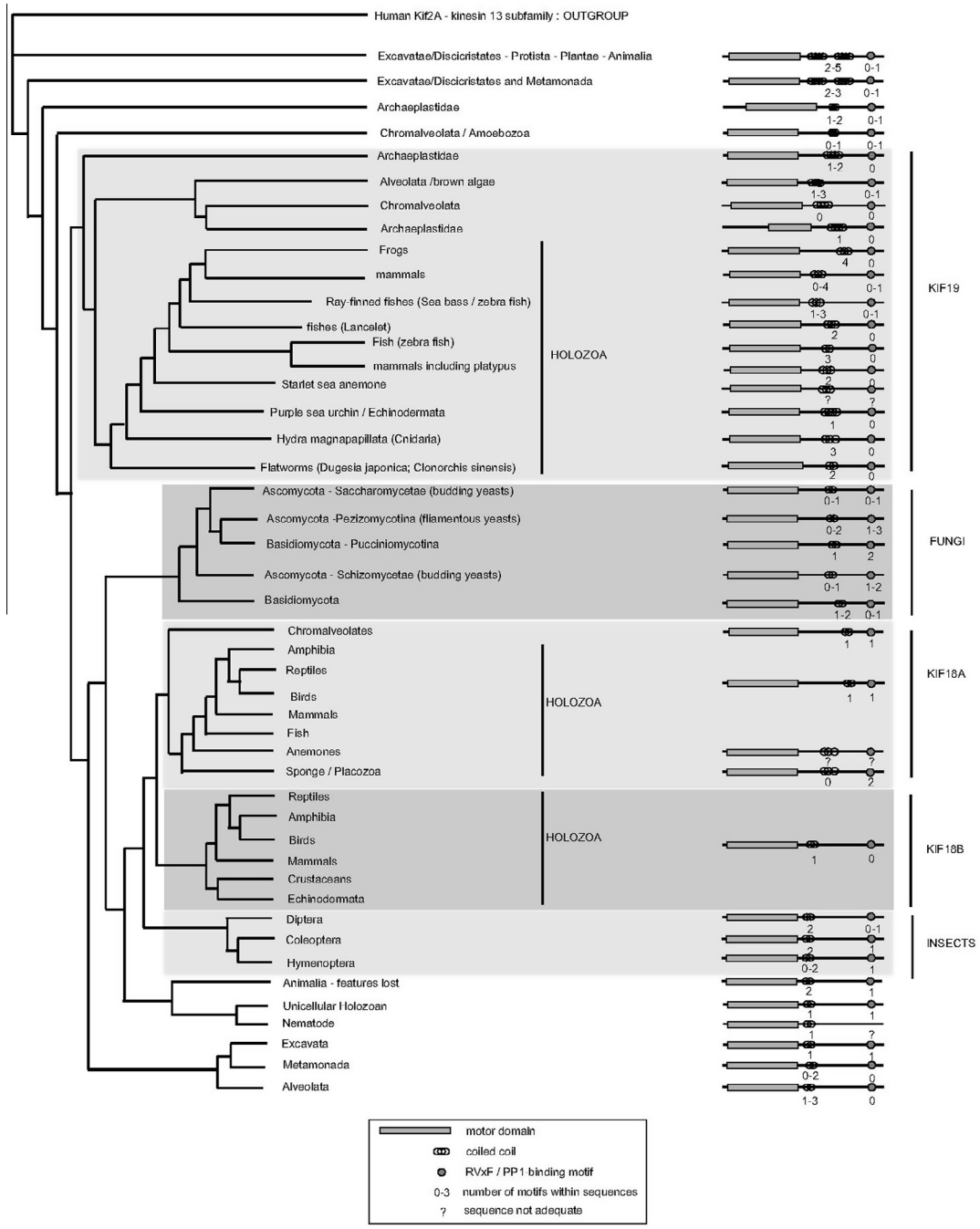

Fig. 2. Rooted neighbor-joining tree of kinesin-8 subfamily and PP1-binding motif distribution. Kinesin-8 sequences were obtained and aligned with ClustalX and Genedoc. A neighbor-joining tree was generated with full length sequences and bootstrapped according to standard parameters. Tree was rooted with the kinesin-13 sequence HsKIF2A and condensed to highlight the evolutionary relationships. Full tree plus putative dock sites are shown in Fig. S2. Motor domains, coiled-coil and PP1-binding motifs were identified (http://elm.eu.org/) and indicated alongside the tree. Numbers underneath reflect the numbers of motifs identified (see Fig. S3). Question marks indicate a lack of publicly available sequences.

variation in the tandem PP1-binding motifs, suggesting the Pezizomycotina were subject to evolutionary pressure yet mostly maintained both motifs, again indicative of functional significance of the PP1-binding motif in motor proteins.

3.4. KIF18A partially co-localizes with PP1 at kinetochore microtubules during mitosis

The proper orientation of chromosomes on the metaphase plate is dependent on KIF18A, which has been shown to suppress kinetochore-microtubule plus-end dynamics [15]. KIF18A localizes to the plus-end MTs during metaphase and with its intrinsic depolymerizing activity helps maintain MT length essential for stable MT-kinetochore interactions.

Knowledge of the biological function of human KIF18A is more limited than its *S. pombe* homolog Klp5/6 and has thus far concentrated on its motor protein capacities [12,16]. We therefore studied the localization of KIF18A relative to PP1 in mitosis by transiently transfecting mCherry-KIF18A into HeLa$^{GFP\text{-}PP1\gamma}$ cells. KIF18A has been shown to localize near the plus ends of kinetochore microtubules during metaphase, flanked by markers for the outer kinetochore (Hec1) and the kinetochore spindle (HURP) [16,17]. Here (Fig. 3) we show that mCherry-KIF18A and GFP-PP1γ partially co-localize at the outer kinetochore region in HeLa$^{GFP\text{-}PP1\gamma}$

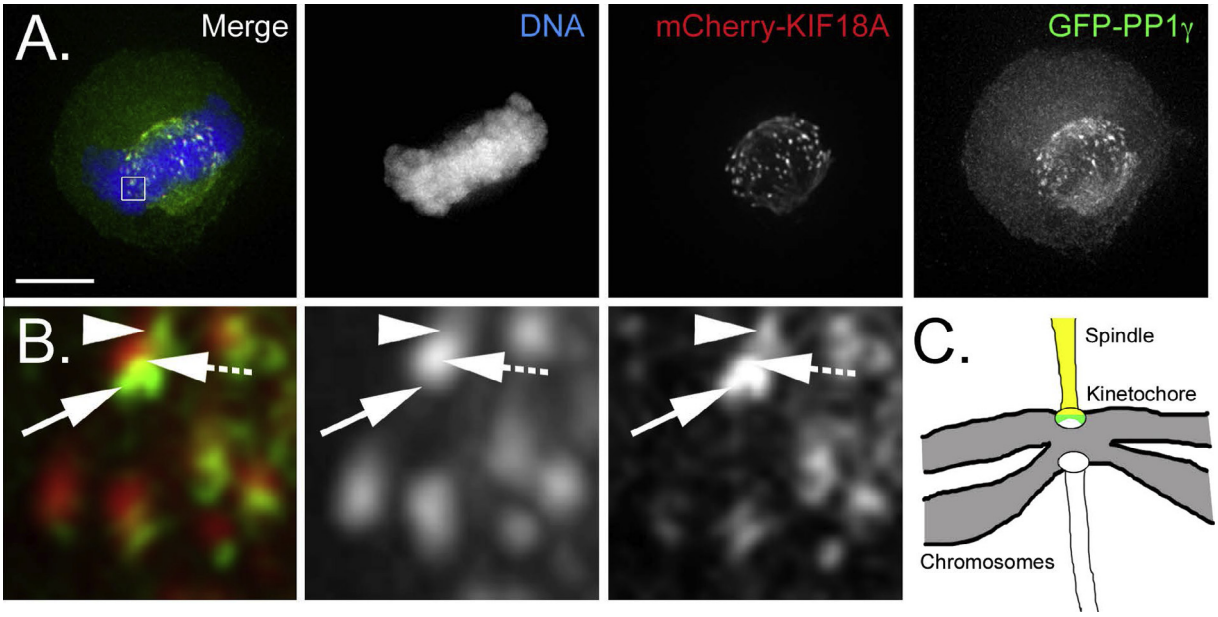

Fig. 3. Partial overlap of KIF18A and PP1γ localization during metaphase. HeLa$^{GFP\text{-}PP1\gamma}$ cells were transiently transfected with mCherry-KIF18A. Panel A shows the localization patterns of GFP-PP1γ (green) and mCherry-KIF18A (red) in a representative metaphase cell that was also stained with Hoechst 33342 to visualize chromosomes (blue). The left panel (merge) shows an overlay of all 3 panels. Scale bar is 10 μm. There is partial colocalization of KIF18A (red) and PP1γ (green) on spindles and at the outer kinetochore, as indicated by regions that appear yellow. The region indicated by the white box has been enlarged in Panel B, to more clearly demonstrate the partial overlap of KIF18A and PP1γ at the outer kinetochore. The DNA signal has been removed for clarity. The arrowhead points to the spindle and the arrow to the kinetochore. A distinct region of overlap can be observed at the outer edge of the kinetochore (hashed arrow). The diagram shown in Panel C depicts the relevant structures and the areas in which colocalization of KIF18A and PP1γ (yellow) is observed. There is a remnant pool of outer kinetochore-associated PP1γ (green), proximal to the chromosome region, that remains distinct from KIF18A. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

metaphase cells. This suggests a potential novel regulatory role of KIF18A in targeting a pool of PP1γ to the outer kinetochores.

Recently, the functional significance of the interaction between the kinesin KIF18A and PP1 was established. KIF18A is a substrate for the kinetochore-localized pool of PP1. PP1 antagonizes CDK1 phosphorylation at residues S674 and S684 in KIF18A, which is important for inhibiting chromosome movements at the metaphase plate thereby aiding in chromosome congression [13].

In summary, we have confirmed the interaction between KIF18A and PP1 in human cells from interphase through to mitotic onset. The PP1-binding motif within the kinesin-8 subfamily is the most widely distributed of all PP1-motifs in the respective kinesin subfamilies. Moreover, despite a highly complex superfamily with numerous gene duplications and secondary losses, the kinesin-8 members and their motif distribution, inclusive of the PP1-binding motif, is remarkably conserved, underscoring the functional importance of this motif.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.09.105.

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