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Alberto Elías-Villalobos[1], Philippe Fort[1], and Dominique Helmlinger[1]

[1]CRBM, CNRS, University of Montpellier, Montpellier, France

*Corresponding author: Dominique Helmlinger

Phone: +33-434-35-95-51

Fax: +33-434-35-94-10

Email: dhelmlinger@crbm.cnrs.fr

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ABSTRACT

Phosphorylation by protein kinases is a fundamental mechanism of signal transduction. Many kinase families contain one or several members that, although evolutionarily conserved, lack the residues required for catalytic activity. Studies combining structural, biochemical, and functional approaches revealed that these pseudokinases have crucial roles *in vivo* and may even represent attractive targets for pharmacological intervention. Pseudokinases mediate signal transduction by a diversity of mechanisms, including allosteric regulation of their active counterparts, assembly of signaling hubs, or modulation of protein localization. One such pseudokinase, named Tra1 in yeast and TRRAP in mammals, is the only member lacking all catalytic residues within the PIKK family of kinases. PIKKs are related to the PI3K family of lipid kinases, but function as Serine/Threonine protein kinases and have pivotal roles in diverse processes such as DNA damage sensing and repair, metabolic control of cell growth, nonsense mediated decay, or transcription initiation. Tra1/TRRAP is the largest subunit of two distinct transcriptional co-activator complexes, SAGA and NuA4/TIP60, which it recruits to promoters upon transcription factor binding. Here, we review our current knowledge on the Tra1/TRRAP pseudokinase, focusing on its role as a scaffold for SAGA and NuA4/TIP60 complex assembly and recruitment to chromatin. We further discuss its evolutionary history within the PIKK family and highlight recent findings that reveal the importance of molecular chaperones in pseudokinase folding, function, and conservation.

Introduction.

A pseudokinase is defined as a kinase paralog that lacks essential catalytic residues and is thus predicted to have no or weakened phosphotransfer activity. Similar to other pseudoenzymes, pseudokinases have been first considered as evolutionary remnants of their active counterparts. However, analysis of their phylogenetic distribution and conservation revealed their prevalence across all kingdoms of life and in many distinct kinase families. Furthermore, their evolutionary conservation outside of catalytic residues clearly argue for important functional roles [1,2]. Pseudokinases have finally received increased attention over the past two decades. Their study provided novel insights into the function of catalytically active kinases, but also revealed their specific roles in many fundamental processes.

Structural, biochemical, and genetic evidence indicate that pseudokinases perform critical non-enzymatic functions in signaling pathways. Their protein-protein interaction domains can allosterically regulate the activities of cognate kinases or other enzymes, compete for substrate binding, scaffold the assembly of signaling complexes, or modulate protein trafficking and localization. Additionally, some pseudokinases with noncanonical catalytic residues show residual activity or ATP binding, which can have a specific regulatory function (for more comprehensive reviews, see [3–8]). Adding to this diversity of mechanisms, recent work showed that the SelO pseudokinase is an active enzyme that uses ATP to transfer AMP to specific substrates [9]. It is therefore becoming clear that much remains to be learned from the study of pseudokinases, and more generally pseudoenzymes [10,11]. Finally, their functions are relevant to numerous human diseases, including cancer, and pseudoenzymes represent attractive targets for novel therapeutic strategies [12].

In this mini-review, we will focus on the structure, function, and evolutionary history of one such pseudokinase, called the transformation/transcription domain-associated protein (TRRAP) in mammals or Tra1 in yeast [13,14]. TRRAP is the sole inactive member of a family of atypical kinases, named phosphatidylinositol 3-kinase related kinase (PIKK), which

comprises the catalytic subunit of the DNA-dependent protein kinase (DNA-PK), ataxia-telangiectasia mutated (ATM), ATM and Rad3-related (ATR), target of rapamycin (TOR), and suppressor of morphogenesis in genitalia 1 (SMG1).

The PIKK family of protein kinases.

PIKKs are related to the phosphatidylinositol class of lipid kinases (PI3K), but function as Serine/Threonine protein kinases, mediating signal transduction in diverse biological contexts (for a review on PIKK fucntions, see [15]). DNA-PK is a critical effector during DNA double-strand break repair. ATM and ATR are the central components of the DNA damage checkpoint and are activated in response to various genotoxic stresses. TOR is a central regulator of metabolism, growth, and survival in response to nutrient availability, growth factors, hormones and stress signals. SMG1 mediates the decay of mRNAs with premature stop codons or that were inappropriately spliced. Finally, the only pseudokinase of this family, TRRAP, has essential roles during transcription. Functional studies in different model systems, including yeasts, nematodes, flies, and mice, have established that these distinct functions are conserved across eukaryotes.

Despite these diverse functions, PIKKs are structurally related and share a characteristic domain architecture. All PIKKs are large proteins, which size ranges from approximately 250 to 470 kDa in humans (Figure 1A). Long arrays of α-solenoids, termed Huntingtin, EF3A, PP2A, TOR (HEAT) repeats, precede a region with high similarity between PIKKs and formed by solenoidal TPR repeats, called the FRAP, ATM, and TRRAP domain (FAT). These extended superhelical structural motifs are immediately followed by the highly conserved, PI3K-related, kinase domain and a short C-terminal FATC motif (Figure 1A). Although the catalytic domain of PIKKs is homologous to that of PI3Ks, notable differences exist in the catalytic motifs (Figure 1B). As compared to PI3Ks, PIKKs contain only the Lys residue from the ATP-binding motif VAIK and the Asp residue from the divalent cation-binding motif DFG. Indeed, PIKKs are strict S/T protein kinases with no reported lipid substrates.

In spite of their sequence similarity, PIKKs differ markedly in their structural organization, mostly of their N-terminal repeats, oligomerization, and interaction with regulatory factors [16,17]. For example, TOR constitutively dimerizes and interacts with accessory proteins to form either TOR complex 1 (TORC1) or TOR complex 2 (TORC2), which are defined by the specific incorporation of either regulatory associated protein of MTOR complex 1 (RPTOR) or RPTOR independent companion of MTOR complex 2 (RICTOR), respectively. In contrast, DNA-PK and ATM dimerization is regulatable and activates DNA-PK, whereas it inhibits ATM activity. Finally, the 'pseudoPIKK' TRRAP is always monomeric but part of larger macromolecular assemblies, the Spt-Ada-Gcn5 acetyltransferase complex (called SAGA both in yeasts and mammals) and the nucleosome acetyltransferase of H4 complex (called NuA4 in yeasts and TIP60 in mammals).

The phylogenetic distribution of PIKKs shows variations between clades, both in the identity of PIKKs and in the number of PIKK paralogs (Figure 2A). TOR, ATM, ATR, and TRRAP are ubiquitously distributed across all major eukaryotic lineages, whereas DNA-PK and SMG1 were lost from several lineages. For example, fungal species from the dikarya lineage, which includes the model organism *Saccharomyces cerevisiae*, lost DNA-PK and SMG1 but have two TOR paralogs, Tor1 and Tor2. Similarly, some Taphrinomycetes have acquired two TRRAP paralogs, Tra1 and Tra2, which have non-redundant functions in the model organism *Schizosaccharomyces pombe* [18,19]. DNA-PK is also absent from the Diptera and Nematoda clades, while SMG1 appears absent from the Chlorophyta and Alveolata, which are clades of plants and protists, respectively. Finally, unicellular parasites from the Excavata clade show striking differences to these general principles, most notably the loss of several PIKKs, which is likely related to their parasitic lifestyle and was noted previously [20]. For example, we observe that, except TOR, all PIKKs were lost from *Giardia intestinalis*, while *Trichomonas vaginalis* appear to have 38 TOR paralogs, 3 TRRAP paralogs, and 2 ATR paralogs. Overall, this analysis reveals that the only 'pseudoPIKK',

Tra1/TRRAP, is conserved throughout all eukaryotic clades and is therefore probably an ancestral member of the PIKK family with important cellular functions.

The Tra1/TRRAP pseudokinase.

Although Tra1/TRRAP is enzymatically inactive, it shows the large size and typical domain architecture of all active PIKKs (Figure 1A). Detailed analysis of Tra1/TRRAP PI3K-like kinase domain indicates that all orthologs and paralogs have lost the three motifs that are essential for enzymatic activity [13,19]. These include the ATP-binding motif VAIK, the catalytic motif HRD, and the divalent cation-binding motif DFG (Figure 1B,C). We note that residues from the catalytic motifs are not conserved across different clades, further arguing that Tra1/TRRAP does not possess enzymatic activity (Figure 1C). In addition, a phylogenetic tree of PIKKs from all major eukaryotic clades confirm that TRRAP is likely an ancestral pseudo-enzyme because its PI3K-like kinase domain diverges more than that of active PIKKs (Figure 2B). Examination of the branch points of this tree suggests that TRRAP is more closely related to DNA-PK than other PIKKs and might have thus originated from its early duplication. However, this hypothesis is not fully supported by a bootstrap analysis and statistical calculations (Figure 2B).

Two recently published studies brought unprecedented insights into the structure of Tra1 and the topological organization of its domains, in particular due to advances in cryo-electron microscopy (EM) approaches [21,22]. Both structures identified three distinct regions within the N-terminal array of HEAT repeats, named the ‘Finger’, ‘Ring’, and ‘Clasp’ domains, which fold into an α-solenoid superhelical structure. The remaining FAT, kinase, and FATC domains form a globular region, termed ‘Head’, which is often referred to as ‘FATKIN’ in other PIKKs and adopts a conformation that is highly conserved between them [17,23]. In contrast, the topology of the HEAT domain is much more variable between PIKKs. Interestingly, the Tra1 HEAT domain adopts a ‘diamond ring’ conformation, which is similar to that of DNA-PK [24], despite apparent low sequence similarity but consistent with their phylogenetic relatedness.

The Tra1/TRRAP 'pseudoPIKK': a protein interaction hub for activator targeting.

Early work hypothesized that the function of Tra1/TRRAP depends on its extensive protein interaction surfaces rather than its pseudokinase domain, similar to a few other pseudokinases such as the Integrin-linked kinase (ILK) or the Tribbles (TRIB) pseudokinases. Although many studies confirmed the importance of protein-protein interaction in Tra1/TRRAP function, mutational analyses of *S. cerevisiae* Tra1 indicated that its pseudokinase domain has also critical roles *in vivo* [25–27]. Similarly, recent work established that the atypical sequence of the TRIB pseudokinase domain relates to an important functional fold, whose plasticity modulates substrate ubiquitination or assembly of signaling modules [28–30].

TRRAP was originally identified as an interacting partner of the c-MYC and E2F transcription factors and is essential for their oncogenic activities during transformation [13]. Since this discovery, numerous biochemical and genetic studies in different organisms established that a diverse range of activators require Tra1/TRRAP to initiate transcription [26,31–38]. Several elegant studies have demonstrated *in vivo*, physical interaction between Tra1 and the transactivation domain of activators [39–44]. Mapping the regions of Tra1 that are involved suggests that its HEAT domain is the main interaction surface for several transcription factors, including Gal4, Gcn4, and Rap1 in *S. cerevisiae*, or c-MYC and p53 in mammalian cells. Indeed, the HEAT domain forms a large exposed surface on Tra1, even when integrated within the SAGA or NuA4 complexes [22,45]. However, the molecular details by which activators interact with Tra1 remains elusive and might involve dynamic, low specificity mechanisms, analogous to Gcn4-Mediator interaction [46]. In addition, whether distinct activators can simultaneously bind to one Tra1/TRRAP molecule remains to be demonstrated, but is conceivable because activators selectively target distinct, non-overlapping regions distributed across the HEAT domain [26,38].

Scaffolding role of the Tra1/TRRAP 'pseudoPIKK' within transcription complexes.

Tra1/TRRAP is predominantly found within either one of two distinct co-activator complexes, SAGA and NuA4/TIP60 [14,47–49]. Both co-activators are highly conserved regulators of transcription initiation and are large multimeric complexes with modular organization (for reviews on SAGA and NuA4/TIP60, see [50–52]). SAGA carries histone H3 acetylation (HAT) and histone H2B de-ubiquitylation (DUB) activities, and modulates the recruitment of the TATA-box binding protein (TBP) to core promoters. Yeast NuA4 acetylates histone H4, H2A, and the histone variant H2A.Z, while the mammalian homologous complex, TIP60, also contains an ATPase subunit, P400, which catalyzes H2A.Z deposition. Altogether, Tra1/TRRAP large size, lack of catalytic activity, and ability to interact with many transcription factors suggested that its primary function during transcription is to scaffold and recruit these complexes to specific promoters.

Recent work from our group have clarified this model, at least in fission yeast. Indeed, *S. pombe* provides a unique opportunity to study Tra1/TRRAP function because, in marked contrast with *S. cerevisiae* and mice, a *tra1*Δ deletion mutant is viable in *S. pombe* [53]. We demonstrated that Tra1 is not required for viability in *S. pombe* because its genome has two paralogous genes, *tra1*+ and *tra2*+, and each has non-redundant roles that are specific for SAGA or NuA4, respectively [19,54]. Phylogenetic analyses indicate that these paralogs result from a duplication of a single gene in the ancestor of the *Schizosaccharomyces* lineage, suggesting that each paralog diverged such that Tra1 and Tra2 are specific for each complex in *S. pombe* [19]. Recently, we observed that the conditional loss of Tra2 disrupts NuA4 integrity and affects cell viability, indicating that Tra2 does indeed scaffold the assembly of the entire NuA4 complex [55]. In marked contrast, we showed that Tra1 has no scaffolding function within SAGA, but, rather, regulates the expression of a small subset of SAGA-dependent genes and specifically controls the incorporation of the DUB module [55]. Therefore, contrary to its general scaffolding role in NuA4 assembly, Tra1 has specific regulatory roles in SAGA structural organization and activity.

These results are consistent with recent structural studies using cryo-EM and cross-linking coupled to mass spectrometry (CXMS) analyses [22,45,56–58]. Indeed, Tra1 is localized at the periphery of SAGA, which contacts the FAT domain through a surprisingly small and flexible region called the 'Hinge'. We recently demonstrated that Spt20 is the major interacting partner of Tra1 within SAGA, in both *S. pombe* and *S. cerevisiae* [55]. In contrast, Tra1 occupies a more central position within NuA4, which subunits make extensive contacts with the FAT, kinase, and FATC domains, explaining the essential role of Tra1 in NuA4 complex integrity and probably the sensitivity of these domains to mutations *in vivo* [26,27,59,60].

To conclude, integrating structural, biochemical, and functional approaches clearly established that Tra1/TRRAP is a pseudoenzyme acting as a protein-protein interaction hub. Despite having no 'writing' activity, Tra1/TRRAP can 'read' cues from promoter-bound transcription factors and relay this signal by recruiting and/or assembling co-activator complexes. Their activities then modify chromatin and stimulate pre-initiation complex assembly to elicit specific transcriptional responses. To date, little is known about the molecular mechanisms by which Tra1/TRRAP controls SAGA and NuA4/TIP60 activities at specific promoters but these recent results have undoubtedly opened new perspectives.

A specific chaperone machinery links Tra1/TRRAP to active PIKKs.

Despite these advances in our understanding of the functional roles of this pseudokinase, the reason for the evolutionary conservation of a typical PIKK domain architecture in Tra1/TRRAP remained a mystery for years. Work from several laboratories recently provided an unexpected and elegant explanation.

Seminal work from Titia de Lange's group first reported a role for a protein called TELO2 in the stabilization of all six mammalian PIKKs, including TRRAP [61]. TELO2 is the mammalian ortholog of *S. cerevisiae* Tel2, which was identified in the first screen for mutants for shortened telomeres [62]. Concurrently, biochemical analysis of TOR complexes in fission yeast identified a trimeric complex composed of Tel2 and two additional proteins, named Tel

two interacting proteins 1 and 2 (Tti1 and Tti2) [18,63]. All three subunits are conserved between yeasts and mammals and form the Triple-T complex (TTT). Further studies in yeast and mammals established that TTT is a novel HSP90 cochaperone dedicated to PIKK stabilization and assembly into active complexes (Figure 3) [61,64–68]. Numerous functional studies in different organisms implicated TTT in PIKK signaling in response to DNA damage or metabolic stress [61,64,66–72]. Importantly, TTT interacts genetically and physically with Tra1 in yeast [18,19,73–75] and stabilizes TRRAP in human cells [61,66–68]. Accordingly, we recently established that, in fission yeast, Hsp90 and TTT promote the *de novo* incorporation of Tra1 into SAGA and of Tra2 into NuA4 [55].

Altogether, these findings have two important implications. First, Tra1/TRRAP, the only 'pseudoPIKK', shares a dedicated chaperone machinery with active PIKKs for its folding and assembly into larger, multimeric complexes (Figure 3). We propose that the requirement of PIKKs for a specific cochaperone explains the selection pressure on the sequence and domain organization of Tra1/TRRAP, despite the divergence of its PI3K-like kinase domain. Supporting this possibility, analysis of the phylogenetic distribution of TELO2, TTI1, and TTI2 indicates that both TELO2 and TTI1 are ancient proteins, because orthologs were found in the genomes of species representative of all major eukaryotic clades, similar to PIKKs (Figure 2A). Interestingly, TTI2 was lost from several lineages, including Diptera and Nematoda, suggesting that its function might not be strictly essential.

Second, although PIKKs are implicated in diverse processes, they are all dependent on HSP90 and its cochaperone TTT for their maturation (Figure 3). HSP90 is indeed a pleiotropic chaperone and typically requires a cochaperone to target and fold a particular subset of substrates, named clients (reviewed in [76]). For example, many HSP90 client kinases are recognized and recruited by the CDC37 cochaperone [77]. It is possible that atypical kinase clients, such as PIKKs, require a specific factor for their recruitment and folding by HSP90. Although the exact mechanism by which HSP90 and TTT promote PIKK maturation remains unknown, we propose that their massive size, unique domain

architecture, obligate partner interactions, and substantial structural flexibility necessitate a dedicated chaperone machinery.

We note that TTT might function as a PIKK-specific adapter, rather than a cochaperone, because its binds HSP90 indirectly. Elegant structural and biochemical studies demonstrated that TELO2 phosphorylation is essential for interaction with a complex called R2TP. This multimeric cochaperone is formed by the RuvB-like AAA+ ATPases RUVBL1 and RUVBL2, the PIH1 domain containing 1 protein (PIH1D1), and the RNA polymerase II associated protein 3 (RPAP3), which TPR domain directly contacts HSP90 (for a review, see [78,79]). However, PIH1D1 and RPAP3 orthologs are absent from fission yeast (G. Lledo, B. Pradet-Balade, and D. Helmlinger; unpublished observations) [80], suggesting either that TTT can bind HSP90 directly in some conditions, or that other factors mediate this interaction. One such factor might be the highly conserved WD40 domain-containing protein Asa1, which copurifies with TTT in both *S. cerevisiae* and in *S. pombe* [73]. Interestingly, a recent study suggests that, in *S. cerevisiae*, Asa1 promotes constitutive TTT-dependent stabilization of $Mec1^{ATR}$ and $Tel1^{ATM}$, whereas the R2TP complex replaces Asa1 in response to stress signals [80].

Conclusions

The widespread phylogenetic distribution and high conservation of seemingly inactive enzymes argue for important catalytic-independent functions. Here, we reviewed and discussed our current knowledge on the only inactive member of the PIKK family of atypical kinases. The discovery of a PIKK-specific cochaperone illuminates the importance of folding and structure in the selective pressure exerted on the sequence of pseudokinases during evolution, and perhaps more generally on pseudoenzymes. As summarized below, several important questions are of particular interest for future research on the Tra1/TRRAP 'pseudoPIKK'. The exact roles of Tra1/TRRAP during transcription are still not fully understood and its study promises many more exciting discoveries in the near future.

Perspectives

- Tra1/TRRAP recruitment to chromatin: Our current view is that DNA-bound transcription factors are responsible for Tra1/TRRAP recruitment to chromatin. However, amongst PIKKs, Tra1/TRRAP is phylogenetically and structurally most related to DNA-PK, which can bind DNA directly [81]. The 3.5 nm-wide opening created by the ring-like conformation of the HEAT repeats of yeast Tra1 might accommodate such a large macromolecule [21,22].
- Function of the PI3K-like kinase domain: Although Tra1/TRRAP lost the ability to bind ATP, it might retain the ability of PI3Ks to bind phosphatidylinositol, recognize another phospholipid, or interact with another negatively charged metabolite. Supporting this possibility, a genetic suppressor screen revealed the functional importance of exposed, positively charged residues in the cleft region of the PI3K-like domain of *S. cerevisiae* Tra1 [27].
- Allosteric regulation of chromatin-modifying activities: pseudokinases can function as pseudoscaffolds modulating the availability of substrates to enzymes (reviewed in [82]). To date, no interaction between Tra1/TRRAP and any PIKK has been reported. Rather, we speculate that Tra1/TRRAP might control the HAT, DUB, or ATPase enzymatic activities of SAGA and NuA4/TIP60. In *S. cerevisiae*, specific *tra1* mutants decrease SAGA or NuA4 HAT activities without affecting their integrity or recruitment [25,26]. In *S. pombe*, we found that Tra1 controls the interaction of the DUB module with SAGA [55]. Tra1/TRRAP might regulate these enzymatic activities by an allosteric mechanism, either through conformational changes within the complex or by controlling accessibility to nucleosomal substrates. Interestingly, although the topology of Tra1 is remarkably rigid [21,23], its contact point with the rest of SAGA appears very flexible [22]. Thus, the relative position of Tra1 to the other functional modules of SAGA might be regulated and used to dictate specific regulatory roles.

FIGURE LEGENDS

Figure 1: Domain architecture and structural features of human PIKKs.

(A) Cartoons depicting the domain architecture (top, colored annotations) and structural features (bottom) of all six PIKKs from *Homo sapiens* (from top to bottom): TRRAP, DNA-PK, ATM, ATR, TOR, and SMG1. Residue numbers indicate the limits of each domain, which were defined based on the most recent structures available [17] and multiple alignments.

(B,C) Multiple alignments of the VAIK, DXXXXN, and DFG catalytic motifs from all human PIKKs **(B)** and of the corresponding regions from selected Tra1/TRRAP homologs **(C)**, using Clustal Omega [83]. Residues that are identical or similar to the consensus sequence are shaded in black or grey background, respectively, using Boxshade 3.2. The canonical VAIK, DXXXXN, and DFG motifs are highlighted and are clearly absent from all Tra1/TRRAP orthologs and paralogs. **(B)** Multiple alignment of human ATR (Q13535), ATM (Q13315), TOR (P42345), SMG1 (Q96Q15), DNA-PK (PRKDC, P78527), TRRAP (Q9Y4A5), and one PI3K kinase (PI3KC3, Q8NEB9). **(C)** Multiple alignment of *S. pombe* Tra2 (Q10064), Tra1 (Q9HFE8), *S. cerevisiae* Tra1 (P38811), *Neurospora crassa* Tra1 (Q7S7K6), *Arabidopsis thaliana* Tra1 (F4IPJ1), *Homo sapiens* TRRAP (Q9Y4A5), *Mus musculus* Trrap (Q80YV3), *Danio rerio* Trrap (A0A0R4IPE4), and *Drosophila melanogaster* TRRAP (Nipped-A, Q8I8U7).

Figure 2: Phylogenetic distribution of PIKKs and of the TTT cochaperone.

(A) Conservation of the TTT complex subunits TELO2, TTI1, TTI2, and of the six PIKKs, TRRAP, DNA-PK (PRKDC), TOR, SMG1, ATM, and ATR across Eukaryotes. All TTT subunits are colored in green whereas each PIKK is colored independently. Orthologs that were not found are indicated by X. Numbers in colors indicate the number of paralogs found in a specific species or lineage when more than 2 were detected. [a]Two copies in Taphrinomycotina; [b]only found in Selaginellaceae; [c]low BLAST scores; [d]only in *Phytophthora infestans*; [e]lack the DXXXXN and DFG motifs and are more closely related to the TRRAP cluster.

(B) TRRAP, DNA-PK (PRKDC), TOR, SMG1, ATM, and ATR PIKK subfamilies were already present in early eukaryotes. Gene models encoding PIKK kinase domains were retrieved in the following taxons: V: Vertebrates (*Homo sapiens*, NP_000042; NP_001175; NP_008835; CAC21449; NP_055907; NP_001231509), FA: Fungi Ascomycota (*Schizosaccharomyces pombe*, BAA33817.1; NP_595357; NP_596275; NP_595359; NP_595777; NP_592862), FM: Fungi Mucoromycota (*Bifiguratus adelaidae*, OZJ03251; OZJ02458; OZJ04327; OZJ04505; OZJ06116), A: Amoebozoa (*Dictyostelium discoideum*, XP_640504; XP_643468; XP_640629; XP_640856; XP_635176), AT: Archaeplastida Tracheophyta (*Selaginella moellendorffii*, EFJ08875; EFJ10333; EFJ09668; EFJ31213; EFJ26294; EFJ26034), AC: Archaeplastida Chlorophyta (*Chlamydomonas reinhardtii*, XP_001693670; XP_001701957; XP_001698462; XP_001697578; PNW79003), E: Euglenozoa (*Leishmania major* strain Friedlin, CBZ11901; CAJ08666; CAJ08193; CAJ09256), H: Heterolobosea (*Naegleria gruberi*, XP_002682730; XP_002674657; XP_002674694; XP_002680550; XP_002680058; XP_002682330), P: Parabasalia (*Trichomonas vaginalis*, XP_001583998; XP_001324760; XP_001329568; XP_001317657), D: Diplomonanida (*Giardia intestinalis*, ESU36088). The tree was rooted with PI3K sequences (BAE06077, NP_594699, XP_001689631, XP_001683719, XP_636122). Note that *T. vaginalis* genome encodes additional PIKK sequences (2 ATR, 2 TRRAP and 36 TOR). The phylogenetic tree was deduced from multiple sequence alignment of the kinase/pseudokinase domain and processed by maximum-likelihood (PhyML) and bayesian (MrBayes) analyses. Only nodes of biological importance for PIKK clustering are indicated. Numbers in red represent PhyML bootstrap proportion (in %) and MrBayes posterior probability.

Figure 3: A dedicated chaperone machinery promotes the maturation and assembly of all PIKK kinases.

Shown is a working model of the HSP90-R2TP-TTT chaperone machinery promoting the maturation and/or assembly of PIKKs. See text for details. Their structures are shown either alone or within their respective complexes with, from top to bottom, SMG1 (EMD-2666 [84]),

TOR (PDB ID: 6BCX [85] for TORC1 complex and PDB ID: 5ZCS [86] for TORC2 complex), TRRAP (EMD-3804 [22] for SAGA complex and PDB ID: 5Y81 model from EMD-6816 [45] for the partial NuA4-TEEAA subcomplex), DNA-PK (PDB ID: 5LUQ [24]), ATM closed dimer conformation (PDB ID: 5NP0 [87]), ATR (PDB ID: 5X6O [88] for $Mec1^{ATR}$ in complex with $Ddc2^{ATRIP}$). The dashed lines identify the PIKK. Images were taken either from the RCSB PDB (rcsb.org) and processed using the NGL viewer [89] or directly from the EMDB (emdataresource.org).

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Author contributions

P.F. performed all phylogenetic analyses. A.E.V. and D.H. wrote the manuscript and all authors read and approved the manuscript.

Conflicts of interest

The Authors declare that there are no conflicts of interest associated with the manuscript.

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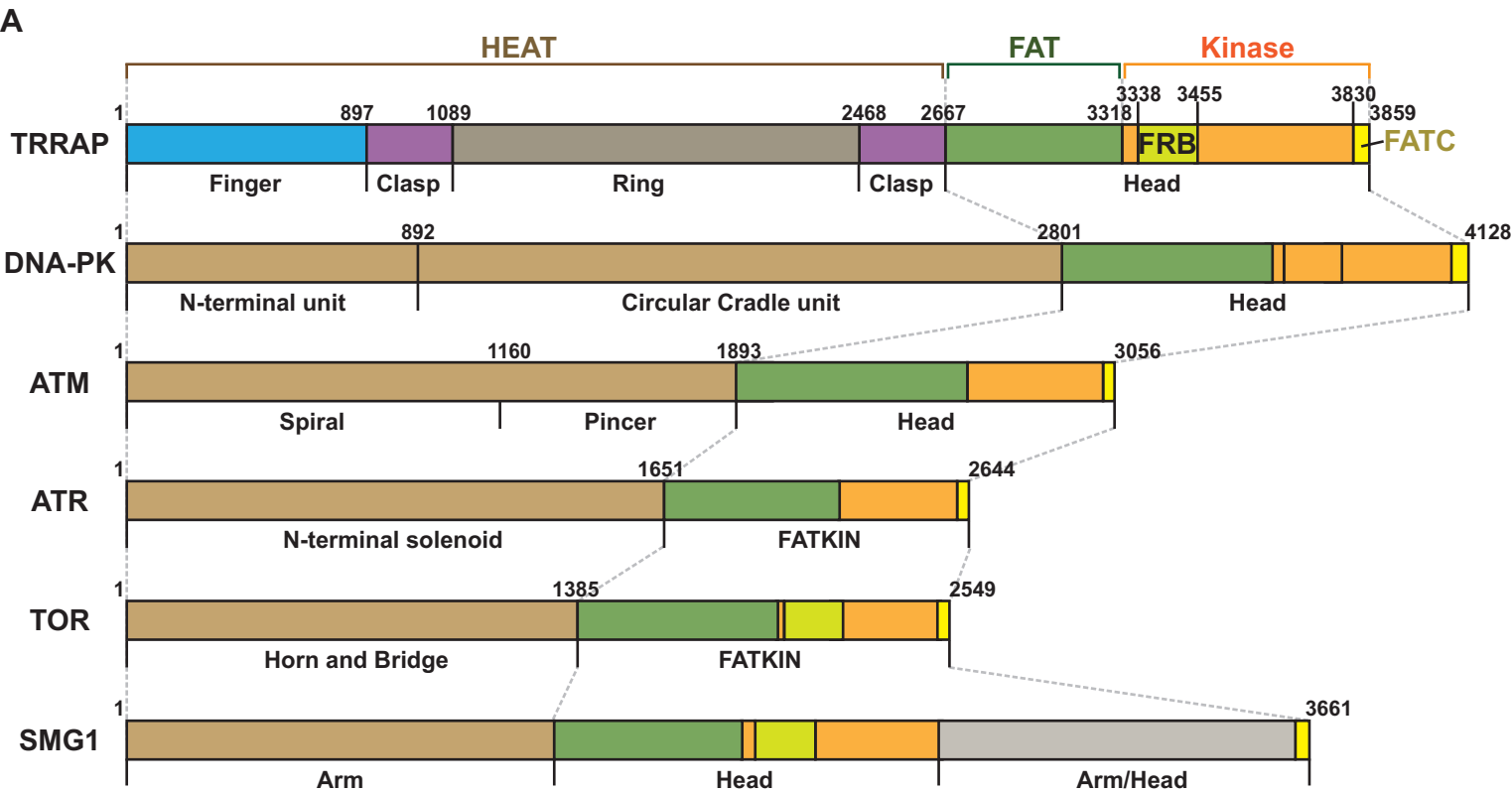
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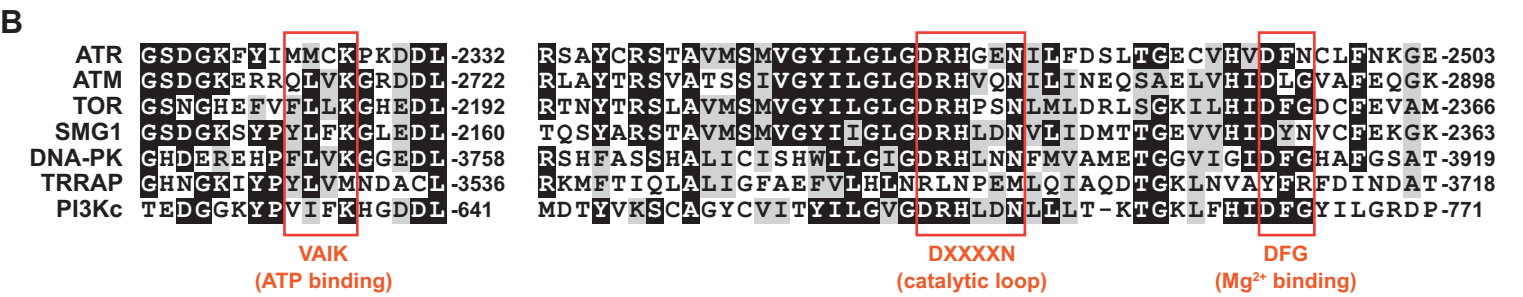
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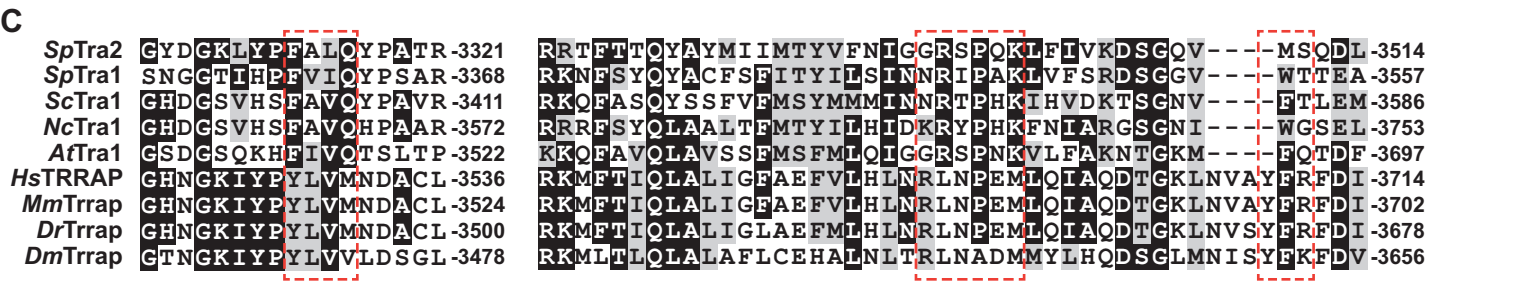
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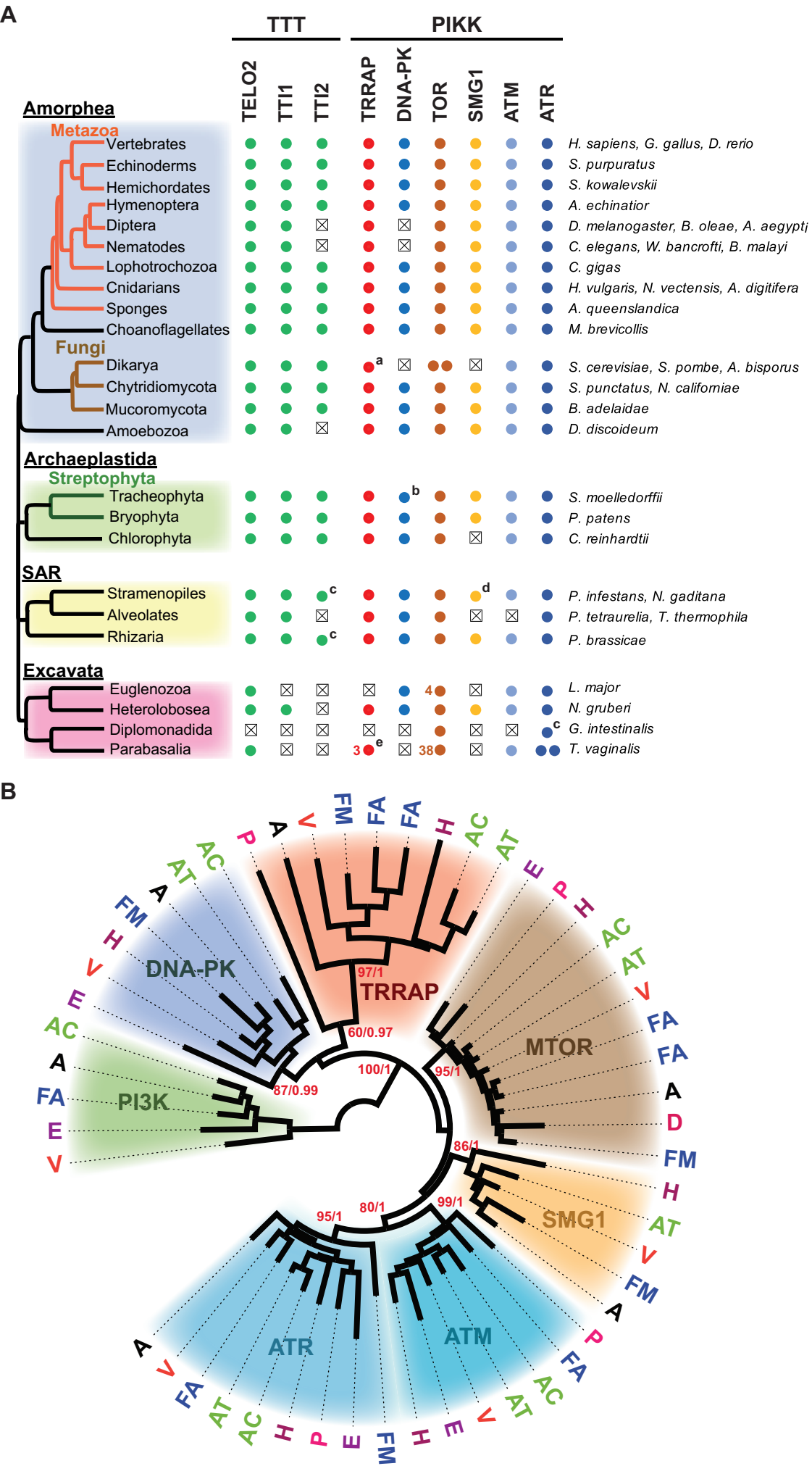
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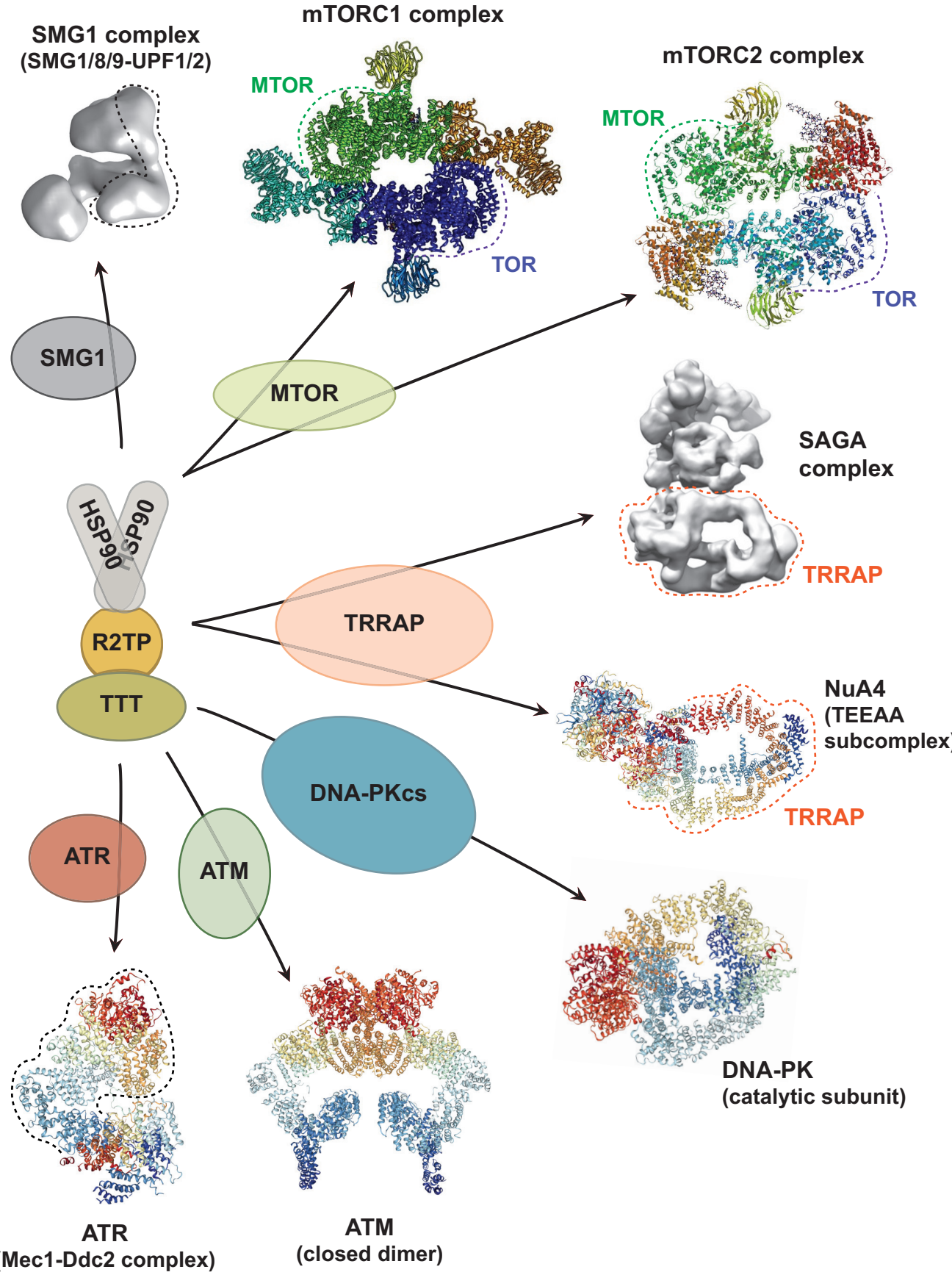
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