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1 **New insights into the evolutionary conservation of the sole PIKK pseudokinase**

2 **Tra1/TRRAP**

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14

1 **ABSTRACT**

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

20

1 **Introduction.**

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

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

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

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

4 **The PIKK family of protein kinases.**

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

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

1 In spite of their sequence similarity, PIKKs differ markedly in their structural organization,
2 mostly of their N-terminal repeats, oligomerization, and interaction with regulatory factors
3 [16,17]. For example, TOR constitutively dimerizes and interacts with accessory proteins to
4 form either TOR complex 1 (TORC1) or TOR complex 2 (TORC2), which are defined by the
5 specific incorporation of either regulatory associated protein of MTOR complex 1 (RPTOR) or
6 RPTOR independent companion of MTOR complex 2 (RICTOR), respectively. In contrast,
7 DNA-PK and ATM dimerization is regulatable and activates DNA-PK, whereas it inhibits ATM
8 activity. Finally, the 'pseudoPIKK' TRRAP is always monomeric but part of larger
9 macromolecular assemblies, the Spt-Ada-Gcn5 acetyltransferase complex (called SAGA
10 both in yeasts and mammals) and the nucleosome acetyltransferase of H4 complex (called
11 NuA4 in yeasts and TIP60 in mammals).

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

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

3 **The Tra1/TRRAP pseudokinase.**

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

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

1 **The Tra1/TRRAP ‘pseudoPIKK’: a protein interaction hub for activator targeting.**

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

11 TRRAP was originally identified as an interacting partner of the c-MYC and E2F
12 transcription factors and is essential for their oncogenic activities during transformation [13].
13 Since this discovery, numerous biochemical and genetic studies in different organisms
14 established that a diverse range of activators require Tra1/TRRAP to initiate transcription
15 [26,31–38]. Several elegant studies have demonstrated *in vivo*, physical interaction between
16 Tra1 and the transactivation domain of activators [39–44]. Mapping the regions of Tra1 that
17 are involved suggests that its HEAT domain is the main interaction surface for several
18 transcription factors, including Gal4, Gcn4, and Rap1 in *S. cerevisiae*, or c-MYC and p53 in
19 mammalian cells. Indeed, the HEAT domain forms a large exposed surface on Tra1, even
20 when integrated within the SAGA or NuA4 complexes [22,45]. However, the molecular details
21 by which activators interact with Tra1 remains elusive and might involve dynamic, low
22 specificity mechanisms, analogous to Gcn4-Mediator interaction [46]. In addition, whether
23 distinct activators can simultaneously bind to one Tra1/TRRAP molecule remains to be
24 demonstrated, but is conceivable because activators selectively target distinct, non-
25 overlapping regions distributed across the HEAT domain [26,38].

26 **Scaffolding role of the Tra1/TRRAP ‘pseudoPIKK’ within transcription complexes.**

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

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

1 These results are consistent with recent structural studies using cryo-EM and cross-
2 linking coupled to mass spectrometry (CXMS) analyses [22,45,56–58]. Indeed, Tra1 is
3 localized at the periphery of SAGA, which contacts the FAT domain through a surprisingly
4 small and flexible region called the ‘Hinge’. We recently demonstrated that Spt20 is the major
5 interacting partner of Tra1 within SAGA, in both *S. pombe* and *S. cerevisiae* [55]. In contrast,
6 Tra1 occupies a more central position within NuA4, which subunits make extensive contacts
7 with the FAT, kinase, and FATC domains, explaining the essential role of Tra1 in NuA4
8 complex integrity and probably the sensitivity of these domains to mutations *in vivo*
9 [26,27,59,60].

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

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

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

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

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

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

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

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

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

17 **Conclusions**

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

1 **Perspectives**

- 2 • Tra1/TRRAP recruitment to chromatin: Our current view is that DNA-bound transcription
3 factors are responsible for Tra1/TRRAP recruitment to chromatin. However, amongst
4 PIKKs, Tra1/TRRAP is phylogenetically and structurally most related to DNA-PK, which
5 can bind DNA directly [81]. The 3.5 nm-wide opening created by the ring-like
6 conformation of the HEAT repeats of yeast Tra1 might accommodate such a large
7 macromolecule [21,22].
- 8 • Function of the PI3K-like kinase domain: Although Tra1/TRRAP lost the ability to bind
9 ATP, it might retain the ability of PI3Ks to bind phosphatidylinositol, recognize another
10 phospholipid, or interact with another negatively charged metabolite. Supporting this
11 possibility, a genetic suppressor screen revealed the functional importance of exposed,
12 positively charged residues in the cleft region of the PI3K-like domain of *S. cerevisiae*
13 Tra1 [27].
- 14 • Allosteric regulation of chromatin-modifying activities: pseudokinases can function as
15 pseudoscaffolds modulating the availability of substrates to enzymes (reviewed in [82]).
16 To date, no interaction between Tra1/TRRAP and any PIKK has been reported. Rather,
17 we speculate that Tra1/TRRAP might control the HAT, DUB, or ATPase enzymatic
18 activities of SAGA and NuA4/TIP60. In *S. cerevisiae*, specific *tra1* mutants decrease
19 SAGA or NuA4 HAT activities without affecting their integrity or recruitment [25,26]. In *S.*
20 *pombe*, we found that Tra1 controls the interaction of the DUB module with SAGA [55].
21 Tra1/TRRAP might regulate these enzymatic activities by an allosteric mechanism, either
22 through conformational changes within the complex or by controlling accessibility to
23 nucleosomal substrates. Interestingly, although the topology of Tra1 is remarkably rigid
24 [21,23], its contact point with the rest of SAGA appears very flexible [22]. Thus, the
25 relative position of Tra1 to the other functional modules of SAGA might be regulated and
26 used to dictate specific regulatory roles.

1 **FIGURE LEGENDS**

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

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

7 **(B,C)** Multiple alignments of the VAIK, DXXXXN, and DFG catalytic motifs from all human
8 PIKKs **(B)** and of the corresponding regions from selected Tra1/TRRAP homologs **(C)**, using
9 Clustal Omega [83]. Residues that are identical or similar to the consensus sequence are
10 shaded in black or grey background, respectively, using Boxshade 3.2. The canonical VAIK,
11 DXXXXN, and DFG motifs are highlighted and are clearly absent from all Tra1/TRRAP
12 orthologs and paralogs. **(B)** Multiple alignment of human ATR (Q13535), ATM (Q13315),
13 TOR (P42345), SMG1 (Q96Q15), DNA-PK (PRKDC, P78527), TRRAP (Q9Y4A5), and one
14 PI3K kinase (PI3KC3, Q8NEB9). **(C)** Multiple alignment of *S. pombe* Tra2 (Q10064), Tra1
15 (Q9HFE8), *S. cerevisiae* Tra1 (P38811), *Neurospora crassa* Tra1 (Q7S7K6), *Arabidopsis*
16 *thaliana* Tra1 (F4IPJ1), *Homo sapiens* TRRAP (Q9Y4A5), *Mus musculus* Trrap (Q80YV3),
17 *Danio rerio* Trrap (A0A0R4IPE4), and *Drosophila melanogaster* TRRAP (Nipped-A, Q818U7).

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

19 **(A)** Conservation of the TTT complex subunits TELO2, TTI1, TTI2, and of the six PIKKs,
20 TRRAP, DNA-PK (PRKDC), TOR, SMG1, ATM, and ATR across Eukaryotes. All TTT
21 subunits are colored in green whereas each PIKK is colored independently. Orthologs that
22 were not found are indicated by X. Numbers in colors indicate the number of paralogs found
23 in a specific species or lineage when more than 2 were detected. ^aTwo copies in
24 Taphrinomycotina; ^bonly found in Selaginellaceae; ^clow BLAST scores; ^donly in *Phytophthora*
25 *infestans*; ^elack the DXXXXN and DFG motifs and are more closely related to the TRRAP
26 cluster.

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

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

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

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

8

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7 **Author contributions**

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

10 **Conflicts of interest**

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

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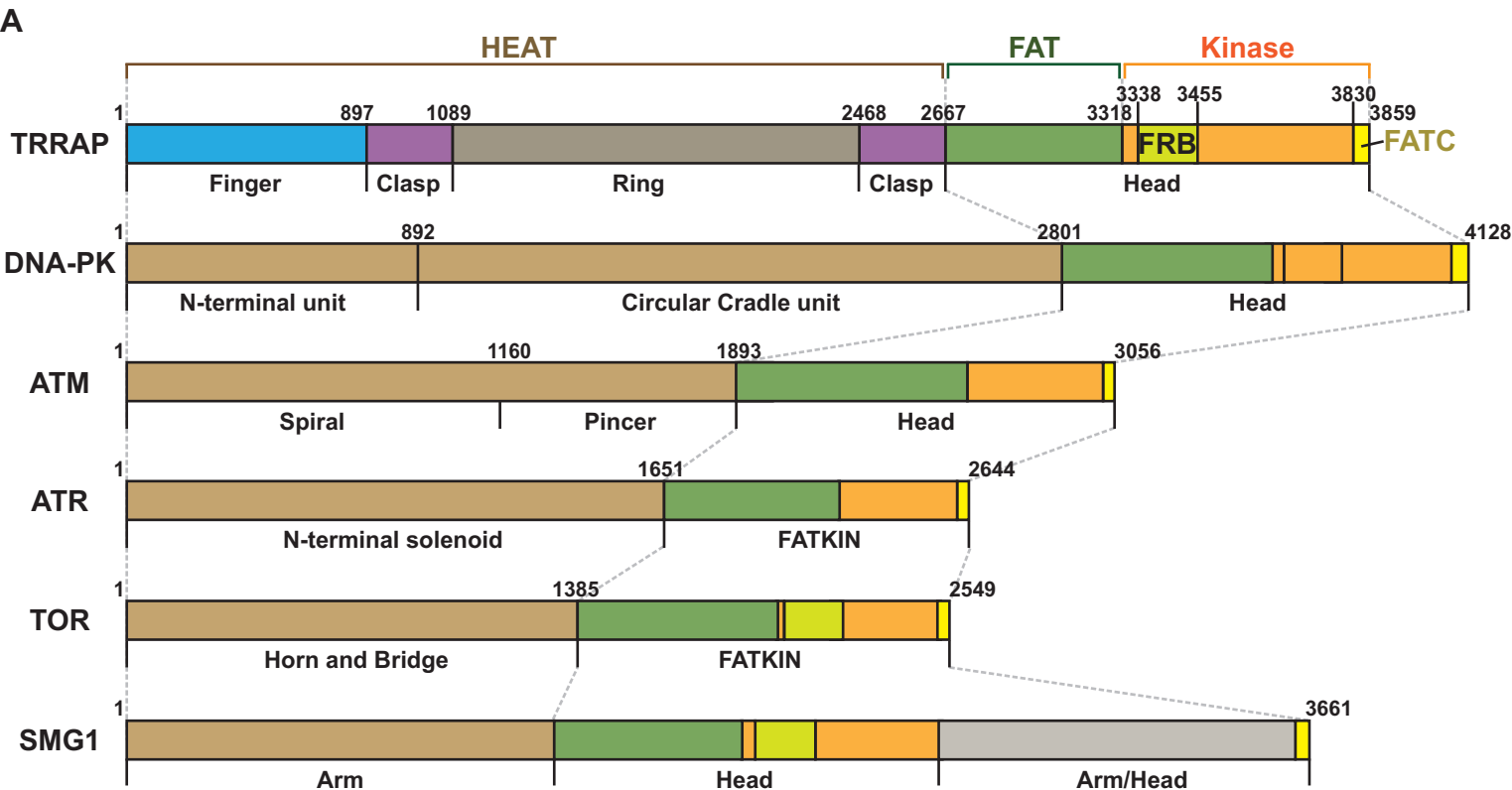
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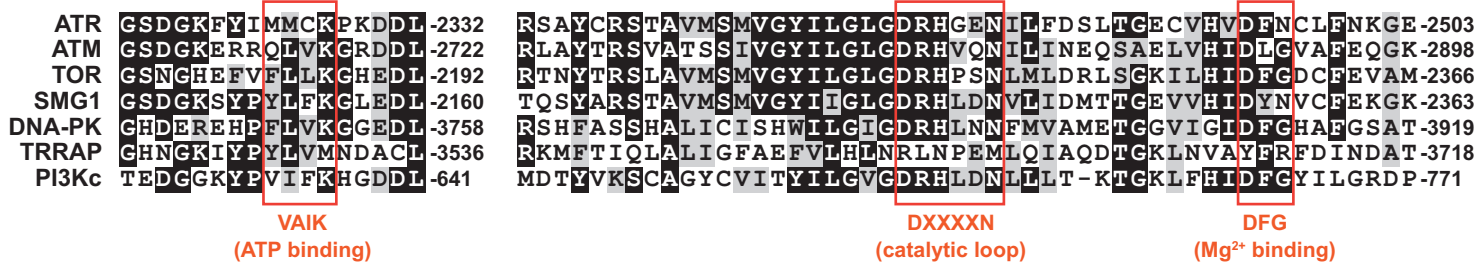
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B



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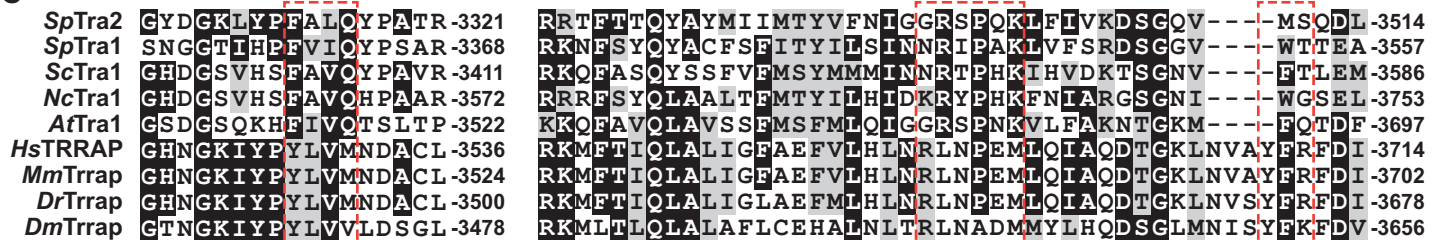


Figure 2

