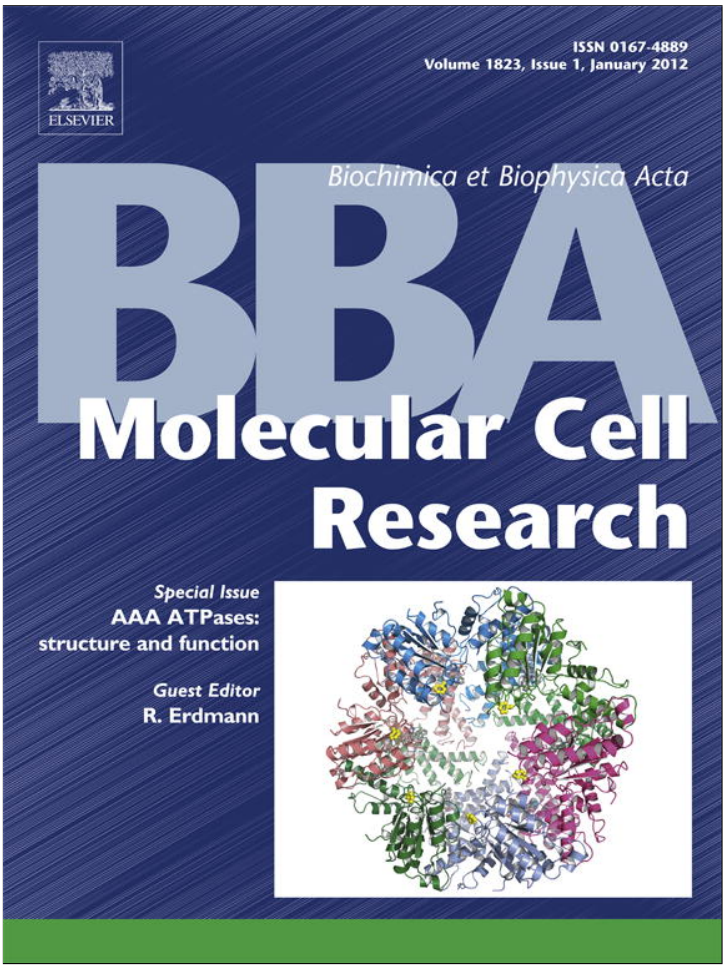

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Review

# The R2TP complex: Discovery and functions☆

Yoshito Kakihara, Walid A. Houry *

*Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada M5S 1A8*

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ABSTRACT

The two closely related AAA+ family ATPases Rvb1 and Rvb2 are part of several critical multiprotein complexes, and, thus, are involved in a wide range of cellular processes including chromatin remodelling, telomerase assembly, and snoRNP biogenesis. It was found that Rvb1 and Rvb2 form a tight functional complex with Pih1 (*P*rotein *i*nteracting with *H*sp90) and Tah1 (*T*PR-containing protein *a*ssociated with *H*sp90), which are two Hsp90 interactors. We named the complex R2TP. The complex was originally isolated from *Saccharomyces cerevisiae* and was, subsequently, identified in mammalian cells. R2TP was found to be required for box C/D snoRNP biogenesis in yeast and mammalian cells. More recently, several studies revealed that the complex is also involved in multiple biological processes including apoptosis, phosphatidylinositol-3 kinase-related protein kinase (PIKK) signalling, and RNA polymerase II assembly. In this review, we describe the discovery of the complex and discuss the emerging critical roles that R2TP plays in distinct cellular processes. This article is part of a Special Issue entitled: AAA ATPases: structure and function.



## 1. Background

Rvb1 and Rvb2 are closely related AAA+ (*A*TPases *a*ssociated with diverse cellular *a*ctivities, Fig. 1A,B) ATPases [1,2] and are homologous to the bacterial RuvB DNA helicase [3,4]. Rvb1 and Rvb2 are highly conserved in eukaryotes and are known by multiple names including RuvBL1, Tip49, Pontin52, Tih1, and TAP54α for Rvb1, and RuvBL2, Tip48, Reptin52, Tih2, and TAP54β for Rvb2 [5–10]. Rvb1 and Rvb2 have been found to be involved in diverse cellular processes such as chromatin remodelling, transcription, telomerase complex assembly, small nucleolar ribonucleoprotein (snoRNP) biogenesis, phosphatidylinositol-3 kinase-related protein kinase (PIKK) signalling, RNA polymerase II (RNAP II) assembly, mitotic spindle assembly, and apoptosis [2,6,7,11–25].

In systematic genome-wide screens for Hsp90-interacting proteins in *Saccharomyces cerevisiae* [26], we identified 627 putative Hsp90 interacting proteins including two proteins which, at that time, were uncharacterized and which we termed Pih1 (*P*rotein *i*nteracting with *H*sp90, YHR034C, Fig. 1A) and Tah1 (*T*PR-containing protein *a*ssociated with *H*sp90, YCR060W, Fig. 1A). Pih1, also known as Nop17, and Tah1 bound tightly to Rvb1 and Rvb2 to form what we termed the R2TP (Rvb1-Rvb2-Tah1-Pih1) complex [26]. Hence, the R2TP complex was initially discovered by our group as an Hsp90-associated multiprotein complex (R2TP-Hsp90 complex) in yeast [26]. Subsequently, a proteomic analysis of human Hsp90 interactors identified the human orthologs of Rvb1, Rvb2, Pih1, as well as, the Tah1 counterpart as Hsp90 interactors [27], and the human R2TP complex was immunopurified from human cell lysates [20]; hence, the R2TP-Hsp90 complex is widely-conserved in eukaryotes. In mammalian cells, the R2TP complex also contains some subunits of the Prefoldin complex namely PFDN2 and PFDN6, as well as Prefoldin-like proteins UXT, RPB5, WDR92/Monad, PDRG1, and URI [18,28–30]. Both the yeast and human complexes were shown to be involved in box C/D snoRNP biogenesis [20,21].

Yeast Pih1 interacts with snoRNP/ribosome biogenesis-related proteins such as Rrp43, a component of the exosome; Nop58, a component of box C/D snoRNP; Nop53, an essential nucleolar protein; and Cwc24, a RING-finger protein related to pre-U3 snoRNA splicing [26,31–33]. The human Pih1, known as PIH1D1 (Fig. 1B), interacts with box C/D snoRNP factors Nop1/fibrillarin, Nop58, Nop56, Tel2 which is a protein required for PIKK protein stability, and WDR92/Monad which is a Prefoldin-like protein containing WD40 repeat [19,20,29,34–36]. The yeast Pih1 is an unstable protein and is prone to be targeted for degradation *in vivo* and to aggregation *in vitro*. However, Pih1 is stabilized by its binding to Hsp90 and Tah1 [21].

Tah1 contains tetratricopeptide repeat (TPR) motifs (Fig. 1A), which are known to mediate the interaction of Hsp90 with its cofactors [37]. By testing the effect of Tah1 on Hsp90 activity, Tah1 was shown to be an Hsp90 cofactor [26]. RPAP3 (FLJ21908), also known as hSpagh, has been identified as the human counterpart of Tah1 in the R2TP complex in human cells [18,20]. However, while yeast Tah1 is 111 residues long and has 2 TPR motifs (Fig. 1A), RPAP3 is 665 residues long and has 6 predicted TPR motifs (Fig. 1B).

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* Corresponding author at: 1 King's College Circle, Medical Sciences Building, Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada M5S 1A8. Tel.: +416 946 7141; fax: +416 978 8548.
*E-mail address:* walid.houry@utoronto.ca (W.A. Houry).

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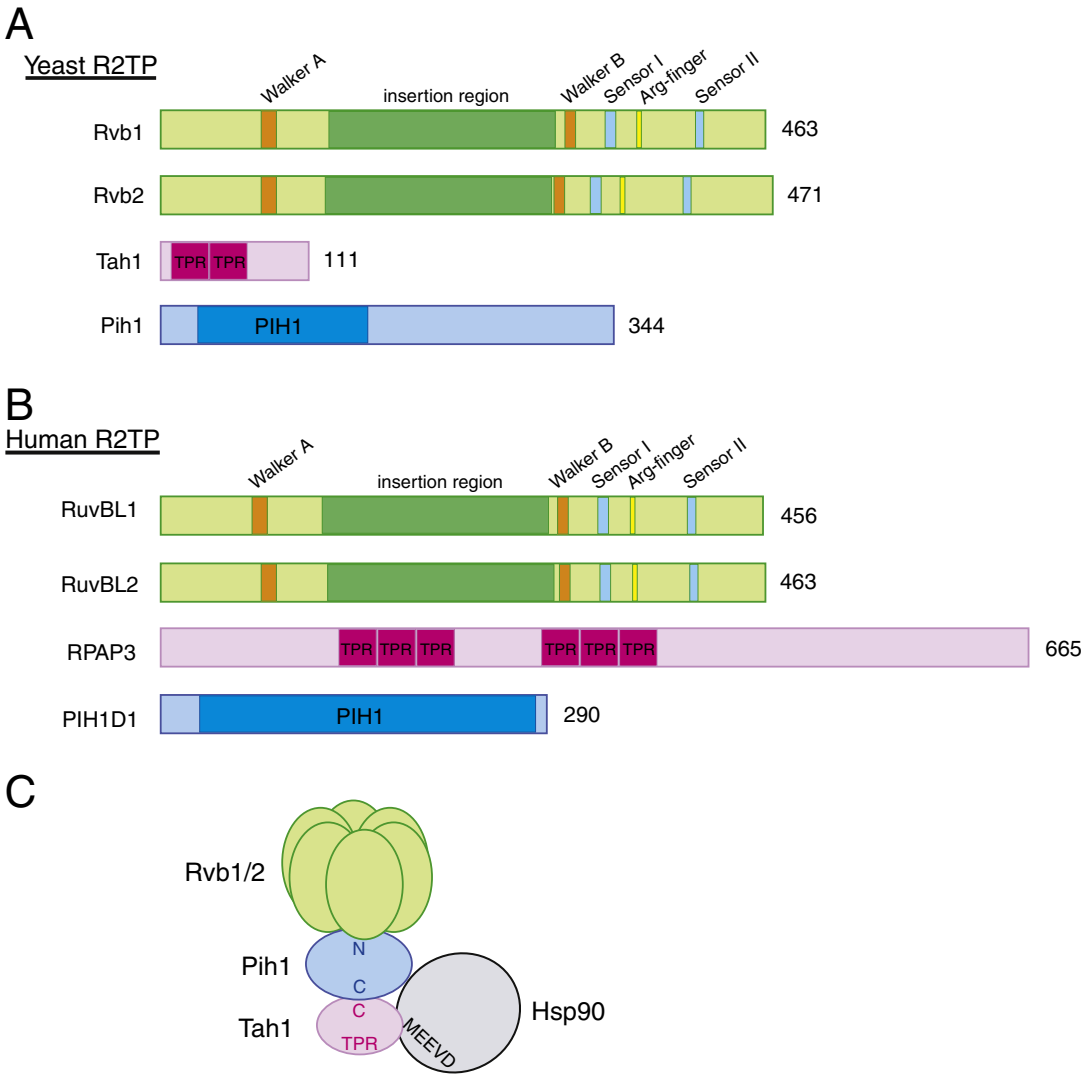


**Fig. 1.** Schematic representation of R2TP. (A) Yeast R2TP proteins: Rvb1, Rvb2, Tah1, and Pih1. (B) Human R2TP proteins: RuvBL1, RuvBL2, RPAP3, and PIH1D1. Yeast Rvb1/Rvb2 and human RuvBL1/RuvBL2 contain the conserved motifs: Walker A, insertion domain proposed to bind DNA/RNA, Walker B, sensor 1, arginine finger, and sensor 2. Yeast Tah1 and human RPAP3 contain TPR motifs. Yeast Pih1 and human PIH1D1 contain the PIH1 domain (also refer to Fig. 3). The domain arrangement of Rvb1, Rvb2, RuvBL1, and RuvBL2 is based on the solved X-ray structure of RuvBL1 [38]. The domain arrangement of Tah1 and RPAP3 is based on the SMART database [83]. While the domain arrangement of Pih1 and PIH1D1 is based on the CDD database [84]. (C) Model of the yeast R2TP complex.

Below, the terms Rvb1, Rvb2, Tah1, and Pih1 will be used when discussing the yeast proteins (Fig. 1A), and the terms RuvBL1, RuvBL2, RPAP3, and PIH1D1 (Fig. 1B) will be used when referring to the mammalian proteins. It should be noted that the human genome contains a PIH1D2 which is 21% identical and 38% similar to PIH1D1, but has not been shown to be part of the R2TP complex.

## 2. Assembly of the R2TP complex

The interactions among the different components of the yeast R2TP-Hsp90 complex have been analyzed [21] (Fig. 1C). The full length Tah1, i.e. the two TPR motifs and the C-terminus (Fig. 1A), interacts with the C-terminal MEEVD motif of Hsp90, while the C-terminus of Tah1 is sufficient to bind Pih1. There is no evidence that Tah1 directly binds the Rvbs. On the other hand, Pih1 binds Tah1, Hsp90, and the Rvbs. More recently, the C-terminus of Pih1 was found to bind to the C-terminus of Tah1 (Fig. 1C).

Yeast Rvb1/Rvb2 and human RuvBL1/RuvBL2 have one AAA+ domain containing the conserved motifs: Walker A, Walker B, sensor 1, arginine finger, and sensor 2 (Fig. 1A). An insertion domain, which is proposed to bind DNA/RNA [38], is present between the Walker A and Walker B motifs (Fig. 1A). The location of the insertion domain within the AAA+ domain is reminiscent of the location of the helical I domain within the AAA+ domain of the unfoldase chaperone HslU/ClpY [39,40]. However, there is currently no molecular understanding as to how nucleotide binding and hydrolysis affects the conformation of this domain or of the complex. In the electron microscopy images obtained by our group of the yeast Rvb1/Rvb2 complex [41], different conformations of the Rvb1/Rvb2 complex were observed in the presence of ADP, ATP, and ATPγS. Hence, it is reasonable to suggest that the insertion domain in the Rvbs might change conformation or orientation in a nucleotide dependent manner.

The oligomeric state of the Rvbs is rather controversial. We proposed that the yeast Rvb1/Rvb2 form a single heterohexameric ring in a 1 to 1 molar ratio [41–43]. Other groups proposed that the Rvbs form a double hexameric ring structure with each ring possibly being a homohexamer of each Rvb [44,45]. In either case, Pih1 seems to act as an adaptor that links Hsp90 and Tah1 to Rvb1/Rvb2 (Fig. 1C). Although the stoichiometry of the different subunits of the R2TP complex is not established, the data currently available to us seems to indicate that Rvb1–Rvb2–Tah1–Pih1 are present at 3:3:1:1

ratio. Fig. 1C provides a schematic of our current understanding of how this complex might be assembled in yeast.

The formation of the human R2TP complex is generally similar to that of the yeast complex, although some differences have been observed since RPAP3 is much larger than yeast Tah1 (Fig. 1A, B). RPAP3 interacts with Hsp90 and PIH1D1 and directly binds to RuvBL1 and, as a result, indirectly associates with RuvBL2 [20]; PIH1D1-RuvBL1 interaction is enhanced in the presence of RuvBL2. Interestingly, the PIH1D1-RuvBL1 and PIH1D1-RuvBL1/RuvBL2 interactions are abolished in the presence of ATP [23].

## 3. R2TP in Box C/D snoRNP Biogenesis

Before the discovery of the R2TP complex, the Rvbs had been identified as essential components of the INO80 and SWR-C ATP-dependent chromatin remodelling complexes. These complexes are involved in transcription, DNA repair, DNA replication, cell cycle checkpoint regulation, chromosome segregation, and telomere maintenance [46]. The Rvbs were also known to be involved in box C/D and box H/ACA snoRNA biogenesis in yeast and human cells [17,47]. Additionally, it had been shown that Pih1 and Rvb1/Rvb2 form a complex that is separate from the INO80 and SWR-C complexes [16]. Shortly after that, Pih1 was identified to interact with Nop58, an essential component of box C/D snoRNP in yeast [31]. Independently of these observations, the R2TP complex was isolated by our group as an Hsp90-interacting complex in yeast, as mentioned above, and was, subsequently, determined to be involved in box C/D snoRNP biogenesis in yeast [21] and human cells [20].

In yeast, disrupting the R2TP complex by deleting the *pih1* gene leads to decreased accumulation of box C/D snoRNAs [21]. Aberrant stoichiometry of the protein components of box C/D snoRNP complex is observed in *pih1*Δ or *hsp90* mutants, suggesting that the R2TP-Hsp90 complex is responsible for box C/D snoRNP assembly (Fig. 2A). Additionally, Rsa1 (Nufip in mammals), a nucleoplasmic protein involved in the assembly of 60S ribosomal subunits [48], has been shown to promote the interaction between box C/D snoRNP and R2TP complexes by specifically binding Snu13, Pih1, Rvb1, and Rvb2 [20].

In mammals, RuvBL1, RuvBL2, Nop56, and Nop58 were identified in nuclear extracts of mouse Taper ascites cells as box C/D snoRNA-associated nucleoplasmic proteins [49,50]. The human R2TP complex directly associates with core box C/D snoRNP proteins and also with other snoRNP accessory proteins [19,20,23,51]. Human Pih1D1 directly associates with fibrillarin/Nop1, Nop58, and Nop56. Human RuvBL1 and RuvBL2 individually interact with all core box C/D snoRNP factors: fibrillarin/Nop1, Nop56, Nop58, and 15.5 K/Snu13. PIH1D1 also interacts with Snurportin1, an $m_3G$ cap-binding import factor [52]; TAF9, a TATA box binding protein (TBP)-associated factor identified as a NOP56 binding protein in a yeast two-hybrid screen [53]; and NUFIP (yeast Rsa1 homolog), a zinc-finger protein that associates with the fragile X mental retardation protein (FMR1) [54]. Also, human RuvBL1/2 complex interacts with BCD1, which is a zinc-finger protein required for box C/D snoRNA accumulation in yeast [55].

It has been shown in human cells that box C/D snoRNP complex assembles hierarchically with the initial formation of box C/D snoRNA-15.5 K/Snu13 subcomplex, which is required for the subsequent association of the other snoRNP components [56]. NOP56 and fibrillarin/Nop1 are able to bind box C/D snoRNA in the absence of Nop58, RuvBL1, and RuvBL2; while RuvBL1 and RuvBL2 bind snoRNA together with Nop58. The depletion of RuvBL1 or RuvBL2 in HeLa cells results in decreased levels of mature box C/D snoRNA [23]. This depletion also affects the trafficking of box C/D snoRNPs to Cajal bodies, which are nuclear compartments through which snoRNPs transit before entering the nucleolus [57].

Taken together, the data indicate that the yeast and human R2TP complexes have essential roles in box C/D snoRNP assembly and translocation from the nucleoplasm to the nucleolus (Fig. 2A).

## 4. R2TP in PIKK signalling

Recently, the R2TP complex has been found to be involved in phosphatidylinositol-3 kinase-related protein kinase (PIKK) signalling pathways [24,58,59] (Fig. 2B). There are six PIKKs in mammals: ataxia–telangiectasia mutated (ATM), ataxia- and Rad3-related (ATR), DNA-dependent protein kinase catalytic subunit (DNA-PKcs), mammalian target of rapamycin (mTOR), suppressor of morphogenesis in genitalia (SMG-1), and transformation/transcription domain-associated protein (TRRAP). These PIKKs function in a wide range of cellular processes [60]. ATM, ATR, and DNA-PKcs are responsible for DNA damage response. mTOR controls cell metabolism and growth in response to mitogenic signals and nutrient availability. SMG-1 is a component of the mRNA surveillance complex involved in nonsense-mediated mRNA decay (NMD) and, hence, regulates the degradation of mRNAs containing premature stop codons. TRRAP is a component of several histone acetyltranferase complexes, such as SAGA, PCAF, and TIP60, and it regulates transcription and functions in DNA repair [61]. Several studies revealed that R2TP-Hsp90 complex plays an essential role in the stability and assembly of PIKKs in mammals [24,58,59].

RuvBL1 and RuvBL2 interact with all PIKKs and knockdown of RuvBL1 or RuvBL2 or treating the cells with Hsp90 inhibitor decreases mRNA levels of ATM, ATR, DNA-PKcs, mTOR, and TRRAP, but not SMG-1 [24]. Furthermore, the RuvBL1/2 complex is required for phosphorylation and activation of direct downstream effectors of ATM, ATR, mTOR, and SMG-1: Chk2, Chk1, p70 S6K, and Upf1, respectively. A function of the RuvBL1/2 complex in the regulation of SMG-1 activity has been characterized [24]. SMG-1 is a component of the SMG1C multiprotein complex which is essential for NMD, composed of SMG-1, SMG-8, and SMG-9. R2TP and Hsp90 have been shown to interact with SMG1C and also with its associated proteins: SMG-10 and RPB5. RuvBL1 and RuvBL2 are needed for SMG-1-mediated Upf1 phosphorylation, and RuvBL1 ATPase activity is essential for this phosphorylation to occur [24]. Also, it has been suggested that this Upf1 phosphorylation results from the sequential remodeling of mRNA surveillance complexes, SURF (SMG1, UPF1, eRF1, and eRF3) and DECID (decay-inducing), which is performed by RuvBL1/RuvBL2. Hence, R2TP-Hsp90 plays an essential role in the formation of the mRNA surveillance complex during NMD.

The interaction between the R2TP complex and PIKKs is mediated by Tel2 [58]. The yeast Tel2 is a DNA binding protein that binds single-stranded telomeric DNA repeats and was originally identified in a screen for mutants with short telomeres [62]. Further studies revealed that Tel2 is involved in diverse cellular processes, such as DNA repair, DNA damage response, and circadian rhythm. [63–66]. Recently, interactions between Tel2 and all six PIKKs were identified in mammals and fission yeast, and it was shown that this interaction is required for the stability of PIKKs [34,67]. It was also shown that the Tel2 complex (Tel2-Tti1-Tti2) interacts with R2TP-Hsp90 complex. Interestingly, Tel2 was found to be constitutively phosphorylated on conserved serine residues 487 and 491 by casein kinase 2 (CK2), a serine/threonine kinase, *in vitro* and *in vivo* [58]. R2TP together with subunits of the Prefoldin-like complex (PFDN2, UXT, URI, RPB5, and WDR92/Monad) interact with the phosphorylated form of Tel2 and this interaction is mediated by PIH1D1. Hence, it is proposed that Tel2 acts as a scaffold protein, assembling R2TP-Hsp90/Prefoldin-like complex with PIKKs to stabilize/assemble the PIKKs, especially mTOR and SMG1 (Fig. 2B).

## 5. R2TP in RNA polymerase II assembly

The R2TP-Hsp90 complex together with Prefoldin-like complex (PFDN2, PFDN6, UXT, RPB5, WDR92/Monad, PDRG1, and URI) have been identified as RNA polymerase II (RNAP II) interacting proteins and have been shown to be involved in RNAP II assembly [28,30] (Fig. 2C). Boulon et al. [28] studied the assembly mechanism of RNAP II in human cells using a combination of quantitative mass

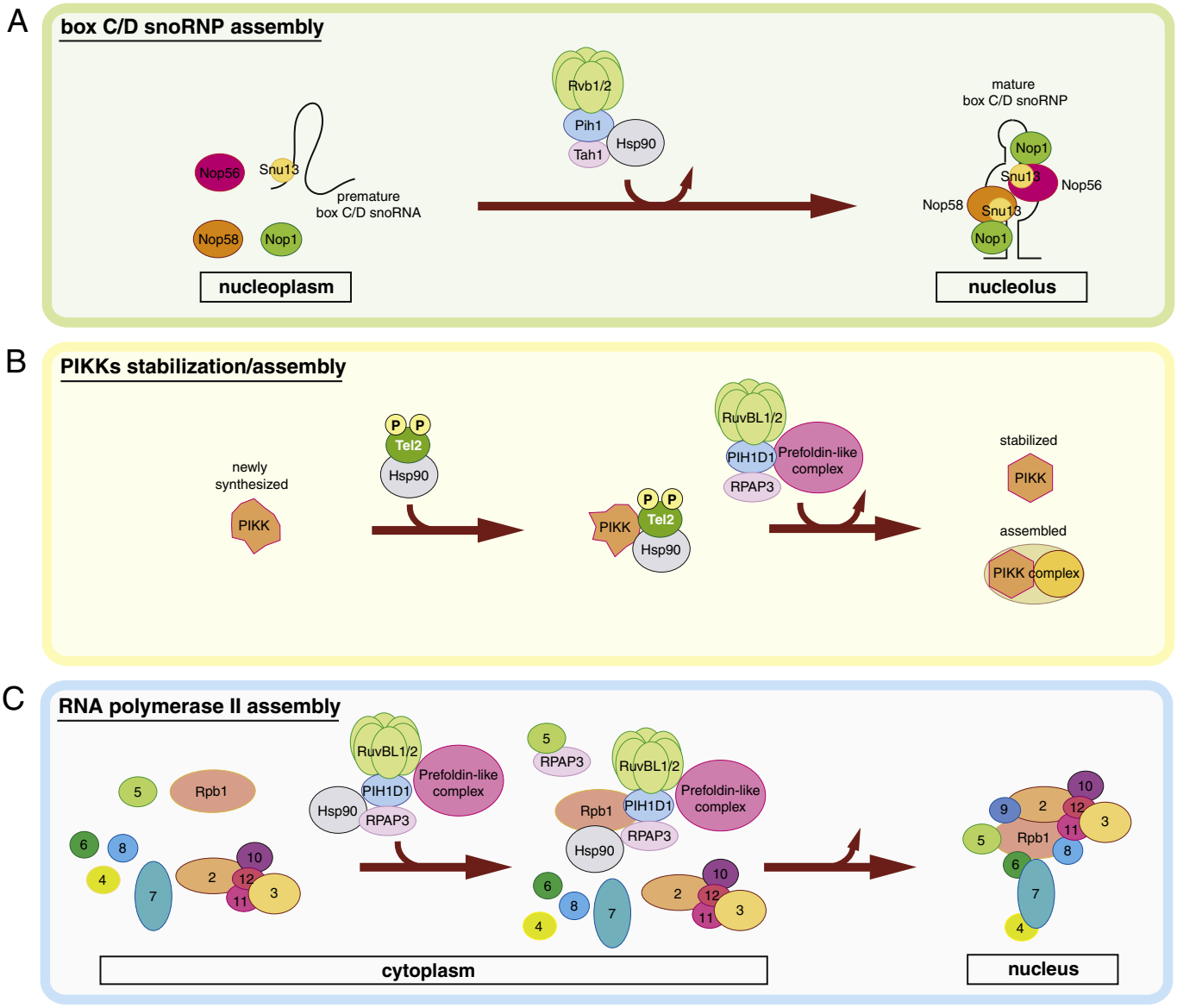


**Fig. 2.** R2TP complex functions. (A) R2TP complex functions in box C/D snoRNP assembly. R2TP complex, together with Hsp90, is required for the assembly of the box C/D snoRNPs and promotes their translocation from the nucleoplasm to the nucleolus. (B) R2TP complex functions in PIKK stability and assembly. Newly synthesized PIKK interacts with Tel2 assisted by Hsp90. Phosphorylated Tel2 then mediates the interaction between PIKK and R2TP-Prefoldin complex to eventually lead to the proper assembly of PIKK. (C) R2TP complex functions in RNA polymerase II assembly. R2TP-Hsp90-Prefoldin complex interacts with unassembled Rpb1. RPAP3 also associates independently with Rpb5. The R2TP-Hsp90-Prefoldin complex promotes the cytoplasmic assembly of RNAP II and its translocation to the nucleus. All the numbers refer to RNAP II subunits Rpb1 to 12.

spectrometry-based proteomic analysis, which makes it possible to measure the relative abundance of a number of proteins, and fluorescence microscopic observations. The authors noted that treatment of cells with α-amanitin and leptomycin B (LMB) leads to accumulation of unassembled Rpb1 in the cytoplasm. α-amanitin specifically binds Rpb1, a large subunit of RNAP II, and induces RNAP II disassembly, transcription arrest, and degradation of Rpb1 *in vivo* [68,69], while LMB is a specific inhibitor of the exportin CRM1 [70]. Furthermore, depletion of any subunit of RNAP II causes the cytoplasmic accumulation of Rpb1. These observations suggest that the assembly of RNAP II occurs in the cytoplasm and that this cytoplasmic assembly is essential for its nuclear import.

Taking advantage of this combinational drug effect, which stabilizes unassembled Rpb1 or Rpb1-containing intermediates of RNAP II in the cytoplasm, quantitative MS analysis was performed on Rpb1 complex immunopurified from cells untreated or treated with α-amanitin and LMB. Treatment with the drugs decreased the association of most of the RNAP II subunits with Rpb1 except for Rpb8, whereas, the R2TP/Prefoldin-like proteins (RPAP3, PFDN2, and UXT) and other RNAP II binding proteins (RPAP2, GPN1, GPN3, and GrinL1A) were found bound to Rpb1. To verify this result, further MS analysis was performed on Rpb1 complexes immunopurified from cells treated and untreated with actinomycin D, which inhibits transcription but does not induce degradation of RNAP II subunits or cytoplasmic accumulation of Rpb1 [28,68]. Under these conditions, less association of the R2TP/Prefoldin-like complex with Rpb1 was detected. These results show that R2TP/Prefoldin-like complex preferentially interacts with unassembled Rpb1 (Fig. 2C). Quantitative MS analysis of RPAP3 complex immunopurified from untreated cells identified the components of the Prefoldin-like complex (PDRG1, PFDN2, PFDN6, URI, UXT, and WDR92) and four of the RNAP II subunits (Rpb1, Rpb2, Rpb5, and Rpb8). Interestingly, it was shown that RPAP3 interacts with unassembled Rpb1 predominantly in the cytoplasm, and that RPAP3 was also found to associate with Rpb5

independently of the Rpb1-containing subcomplex. Depletion of RPAP3 led to destabilization of the cytoplasmic Rpb1. Furthermore, inhibition of Hsp90 activity with geldanamycin (GA) destabilized unassembled Rpb1 that accumulated upon either α-amanitin + LMB treatment or Rpb2 depletion. Based on these experiments, it was concluded that the R2TP-Hsp90/Prefoldin-like complex is required for proper assembly of RNAP II in the cytoplasm by specifically associating with unassembled Rpb1 [28,68]. Fig. 2C shows a simplified schematic of the proposed assembly pathway for RNAP II.

## 6. R2TP complex in apoptosis

RPAP3, RuvBL2, and PIH1D1 of the human R2TP complex have been identified as Monad/WDR92 interacting proteins [35,36]. As mentioned above, Monad/WDR92 is a subunit of Prefoldin-like complex; it is a WD40 repeat protein that was found to be involved in apoptosis [71]. The overexpression of either Monad/WDR92 or RPAP3 in HEK293 cells enhances apoptosis and caspase-3 activation induced by tumour necrosis factor-α (TNF-α) and cycloheximide (CHX), whereas the depletion of RPAP3 significantly reduces the induction of apoptosis, suggesting that Monad/WDR92 and RPAP3 could be modulators of the apoptotic pathway [35,71]. Also, overexpression of RPAP3 promotes UV-induced cell death, while knockdown of RPAP3 decreases cell death. In contrast, knockdown of RuvBL2 enhances cell death upon UV treatment. Furthermore, depletion of PIH1D1 promotes apoptosis and caspase-3 activation induced by doxorubicin in U2OS osteosarcoma human cells [36].

RPAP3 has also been identified to repress NF-κB pathway, which is activated by DNA damage stress and which mediates a cell survival pathway [72]. The activation of the NF-κB is initiated by a signal-induced phosphorylation and subsequent ubiquitination and degradation of IκB (Inhibitor of κB) proteins. This IκB phosphorylation is performed by IKK (IκB kinase). IKK is composed of IKKα, IKKβ, and IKKγ/NEMO (NF-κB essential modulator) [73]. The polyubiquitination of IKKγ/NEMO plays an essential role in the activation of the IKK complex [74]. It was shown that RPAP3 interacts with IKKγ/NEMO and that the overexpression of RPAP3 inhibits the polyubiquitination of IKKγ/NEMO and enhances doxorubicin-induced cell death through the inhibition of NF-κB pathway.

Taken together, the data indicate that RPAP3 and Monad/WDR92 are pro-apoptotic, while PIH1D1 and RuvBL2 are anti-apoptotic, suggesting that the R2TP proteins may have additional separate functions in apoptosis or that each subunit differentially regulates the activity of the R2TP complex [71,72].

## 7. PIH1 family proteins in axonemal dynein assembly

Characterization of Pih1 function in box C/D snoRNP and PIKKs and to some extent RNAP II assembly strongly suggests that it mainly functions as an adaptor to target the R2TP-Hsp90 complex to client proteins. Interestingly, Pih1 adaptor function was also identified for other PIH1 family proteins, namely, Ktu/PF13 and MOT48. These proteins have weak sequence similarity with yeast Pih1 (Fig. 3) and are involved in the assembly of the dynein arms in flagella/cilia [75,76]. Flagella/cilia are highly organized microtubule-based structures that are composed of complex components such as radial spokes, a ring of nine doublet microtubules surrounding a pair of single microtubules called the central pair, and inner and outer dynein arms (IDA and ODA) tightly anchored to the outer doublet microtubules [77]. IDA is required for proper flagellar waveforms, whereas ODA is necessary for highly frequent flagellar beating [78]. Omran et al. [75] found a medaka (*Oryzias latipes*/Japanese killifish) mutant, which they named *ktu* (Kintoun), that shows defects in cilia motility in Kupffer's vesicle, an epithelial sac containing fluid that is required for left-right asymmetry, as well as, defects in sperm motility. This mutant exhibited partial or complete loss of IDAs and ODAs. Positional cloning identified the mutation to be a premature stop codon in a gene encoding 588 amino acids with weak sequence similarity to yeast Pih1. An ortholog of Ktu was identified in human cells (C14orf104). Mutation of human Ktu causes primary ciliary dyskinesia (PCD), which is characterized by a complete loss in the motility of respiratory cilia and sperm flagella due to abnormal axonemal dynein arms [75].

Ktu function has been extensively analyzed in *Chlamydomonas*, which has a Ktu ortholog named PF13 (Fig. 3). PF13 mutant has a paralyzed flagella phenotype caused by impaired formation of IDAs and ODAs. *Chlamydomonas* dynein arms are preformed in the cytoplasm [79,80], and the pre-assembled dynein complex is then transported to the flagellar compartment by intraflagellar transport (IFT) [81,82]. PF13 was found to be required for cytoplasmic pre-assembly of ODA heavy chains with intermediate and light chains, but not for the interaction between intermediate chains and light chains. The mouse ortholog of Ktu (mKtu) was found to interact with one of the intermediate chains and with Hsp70. It is speculated that Ktu/PF13 could function as a co-chaperone of Hsp70 to facilitate the cytoplasmic pre-assembly of dyneins [75]. Recently, another PIH1 family protein in *Chlamydomonas*, MOT48 (Fig. 3), has also been shown to be required for the pre-assembly of axonemal dyneins, especially IDAs [76].

The above observations suggest that PIH1 family proteins are generally responsible for the pre-assembly of different subsets of axonemal dyneins. However, at this point there is no indication whether RuvBL1/2 or Hsp90 also play a role in dynein-related assembly pathways.

## 8. Mechanism of the function of R2TP complex

Although Rvb1/Rvb2 are involved in a number of cellular processes by interacting with different protein complexes, the function of the R2TP complex is more specifically related to maturation/assembly of certain protein complexes such as snoRNP biogenesis, PIKKs signalling, and RNA polymerase II. In general terms, it has been found that Pih1 and/or RPAP3/Tah1 in the R2TP complex specify the target to be acted upon by Rvb1/Rvb2, and that Pih1/Tah1 assist in the interaction between Rvb1/Rvb2 and those targets. In the case of snoRNP and PIKK complex assembly, Pih1 works as an adaptor protein which mediates interaction between the R2TP complex and either box C/D snoRNP or PIKK complexes. In RNA polymerase II assembly, the process is initiated by the interaction of RPAP3/Tah1 with an unassembled subunit of RNA polymerase II. After the association of R2TP complex with its substrates, Rvb1/Rvb2 promote the assembly/maturation of the multiprotein complex by their proposed chaperone activity.

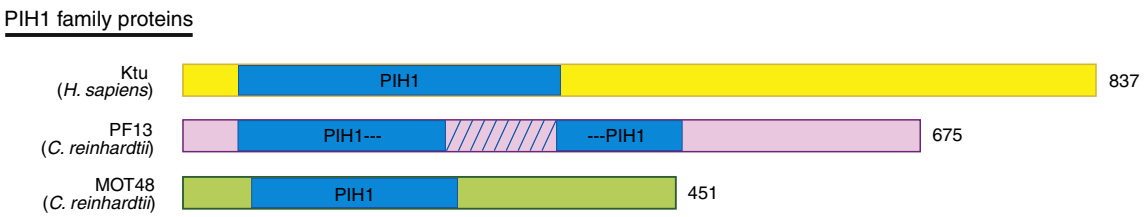


**Fig. 3.** PIH1 family proteins involved in dynein assembly. Domain organization of the PIH1 family proteins Ktu (human), PF13 (chlamydomonas), and MOT48 (chlamydomonas) are shown as obtained using the CDD database [84]. Note that the PIH1 domain of PF13 is interrupted by a low complexity region indicated by the stripes.

Although Rvb1/Rvb2 has demonstrated ATPase and helicase activities, it remains unknown how R2TP assemble the multiprotein complexes and how the motor function of the Rvb1/Rvb2 relates to the assembly processes. Furthermore, this chaperone or chaperone-like activity of the R2TP complex can be further modulated by other chaperones such as Hsp90 and Prefoldin-like complex through direct interactions.

## 9. Conclusion

The R2TP complex was found in a screen for Hsp90 interactors in yeast [26]. Since then, this complex has been shown to be highly conserved in eukaryotes and to be involved in several specific cellular processes including box C/D snoRNP biogenesis, PIKK signalling, and RNAP II assembly (Fig. 2). Its general role seems to be in the assembly of multiprotein/multiprotein-RNA/multiprotein-DNA complexes. Although studies of the R2TP complex in different biological processes have greatly advanced our understanding of its functions, much remains to be learned about the molecular basis of its activity. Future structural and functional studies focused on determining the exact molecular activity of R2TP on its target substrates will be critical in furthering our understanding of this important complex.

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