Table 1. (continued)

| GO term: RNA binding (GO:0003723) | | | |
|---|---|---|---|
| Gene | FPKM 4sU 0 h | FPKM 4sU 4 h | |
| *Nusap1* | 49.76 | 8.38 | Downregulated |
| *Tst* | 6 | 0.87 | |
| *Tnrc6b* | 20.73 | 3.15 | |
| *Kctd12* | 5.49 | 0.77 | |
| *Abtb1* | 37.57 | 4.75 | |
| *Sidt1* | 13.37 | 1.45 | |
| *Hbp1* | 31.77 | 2.92 | |
| GO term: transcription factor activity, sequence-specific DNA binding (GO:0003700) | | | |
| Gene | FPKM 4sU 0 h | FPKM 4sU 4 h | |
| *Crebl2* | 12.79 | 2.43 | Downregulated |
| *Zfp874b* | 15.54 | 2.89 | |
| *Tfdp2* | 3.93 | 0.66 | |
| *Zfp260* | 37.6 | 6.4 | |
| *Zscan2* | 10.77 | 1.76 | |
| *Zfp831* | 4.69 | 0.6 | |
| *Rfx3* | 2.66 | 0.2 | |
| *Hbp1* | 31.77 | 2.92 | |
| *Crebrf* | 31.38 | 2.32 | |
| *Nacc2* | 11.76 | 0.25 | |

[a]Genes that were >fivefold regulated in newly transcribed [4-thiouridine (4sU)-labeled) RNA during T cell receptor (TCR) stimulation of T helper 1 cells [5]. Genes are categorized according to the Gene Ontology (GO) terms transcription factor activity (GO:0003700) or RNA binding (GO:0003723), and are ordered according to their fold changes from the highest upregulated to the highest downregulated gene for each GO category. Gene expression (fragments per kb of transcript per million reads, FPKM) values are shown for unstimulated (0 h) and activated cells (4 h).

>fivefold after 4 h of activation, highlighting several new genes so far not known to play a role in immune cells.

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Forum

# The PAQosome, an R2TP-Based Chaperone for Quaternary Structure Formation

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**The Rvb1–Rvb2–Tah1–Pih1/prefoldin-like (R2TP/PFDL) complex is a unique chaperone that provides a platform for the assembly and maturation of many key multiprotein complexes in mammalian cells. Here, we propose to rename R2TP/PFDL as PAQosome (particle for arrangement of quaternary structure) to more accurately represent its unique function.**

## Discovery, Subunits, and Conservation

Almost 12 years ago, the R2TP (see Table 1 for nomenclature) complex was discovered by Houry and colleagues [1] in a large-scale screen for Hsp90 interactors in *Saccharomyces cerevisiae*. Subsequently, the complex was found to be

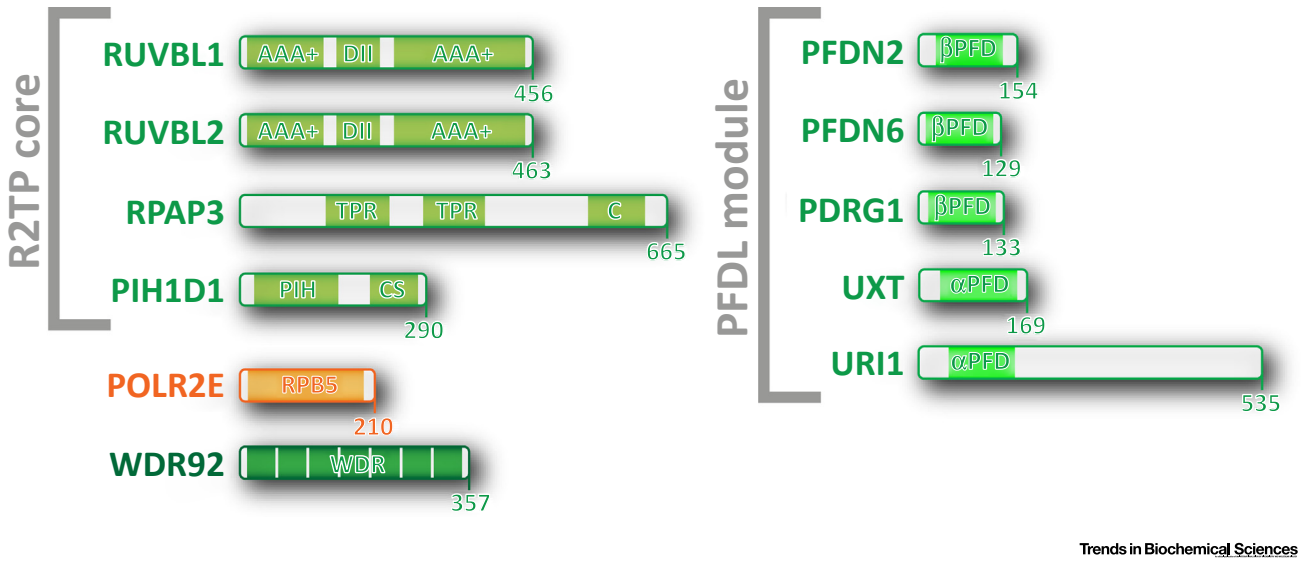


Figure 1. Domain Architecture of the PAQosome Subunits. Abbreviations are given in Table 1.

highly conserved in eukaryotes [2,3]. In yeast, R2TP consists of the AAA+ ATPases Rvb1 (approximately 50 kDa) and Rvb2 (approximately 52 kDa), Pih1 (approximately 39 kDa), and the TPR protein Tah1 (approximately 13 kDa). Human R2TP contains orthologous proteins, named RUVBL1 (Pontin), RUVBL2 (Reptin), RPAP3, and PIH1D1 (Figure 1). Yeast and human R2TP proteins are similar with the exception of human RPAP3, which is larger and more complex than its yeast counterpart (76 kDa vs. 13 kDa, respectively). In mammals, R2TP associates with a PFDL module to form the R2TP/PFDL complex. PFDL consists of two α subunits and four β subunits (Figure 1). The α subunits are UXT and URI1 (also present in yeast as Bud27). The β subunits are PFDN2, PFDN6, and PDRG1 with one of the subunits likely duplicated. PFDL also includes two additional components, the RNA polymerase subunit POLR2E (or RPB5) and WDR92 (or Monad) [4–6] (Figures 1 and 2).

In both the yeast and human R2TP, Tah1/RPAP3 stably associates with Pih1/PIH1D1, and the resulting heterodimer binds Rvb1/2 (RUVBL1/2; Figure 2). Pih1 and Tah1 are not essential in *S. cerevisiae* and homologs of these proteins are absent in some yeasts, fungi, and plants. By contrast, Rvb proteins are found in all eukaryotes and are essential proteins. They are the catalytic components of R2TP, while Tah1/RPAP3 and Pih1/PIH1D1 are believed to function as adaptors or as regulatory proteins. The Rvb proteins form a hexameric ring complex typical for AAA+ proteins, but they contain an additional insertion domain called domain II (DII; Figures 1 and 2). This is a flexible domain that protrudes from the Rvb1/2 ring and can mediate further interactions as well as the dimerization of the Rvb1/2 hexameric ring.

Pih1/PIH1D1 consist of an N-terminal PIH domain that can bind 'DSDD/E' motifs phosphorylated at the serine residue [7], and a C-terminal CS domain that mediates interaction with Tah1/RPAP3 (Figures 1 and 2). Yeast Tah1 contains a TPR domain that binds the C-terminal MEEVD motif of Hsp90, and an unstructured tail that inserts into the Pih1 CS domain [7] (Figure 1). RPAP3 contains two TPR domains (Figures 1 and 2) that can independently bind HSP90 but with different affinities, and has a conserved but uncharacterized C-terminal domain as well as a potential N-terminal domain [7] (Figure 1).

## Proposed Functions and Substrates of R2TP/PFDL

Mammalian R2TP/PFDL was found to interact with ribonucleoproteins of the L7Ae family (box C/D and H/ACA snoRNPs, U4 snRNPs, and telomerase and selenoprotein mRNPs) as well as U5 snRNP, and the available data indicate that R2TP/PFDL acts as an assembly factor for these RNPs [3,6,8]. Mammalian R2TP/PFDL was found to also play essential roles in the biogenesis or stability of PIKKs (ATM, ATR, DNA-PKcs, mTOR, SMG-1, and TRRAP, the latter of which lacks kinase activity) [9–11]. It has been shown that RNA polymerase I, II, and III are also substrates for R2TP/PFDL [5,6]. Finally, it has been found that R2TP/PFDL interacts with all the subunits of the TSC (TSC1, TSC2, and TBC1D7), a tumor-suppressor complex mutated in the tuberous sclerosis tumor syndrome [6,8]. The list of R2TP and R2TP/PFDL clients suggests a role in the assembly of

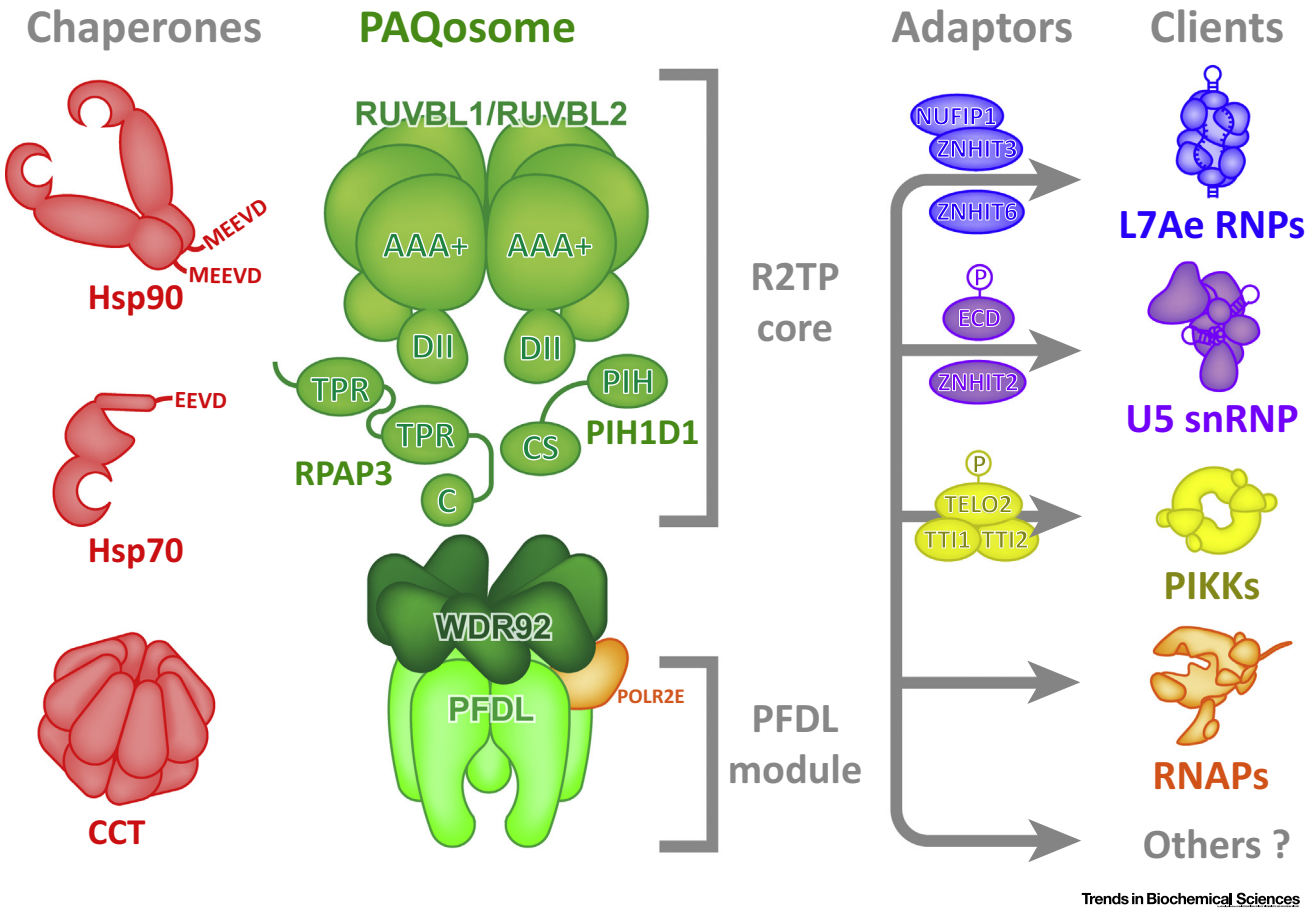


Figure 2. Schematic of the PAQosome Structure, Adaptors, and Clients. Schematic representation of the PAQosome (green), associated chaperones (red), known adaptors (right; on arrows), and client complexes (far right). R2TP core and PFDL module are shown. Note that not all the interactions have been well established or characterized. Abbreviations are given in Table 1.

Table 1. Nomenclature.

| | |
|---|---|
| αPFD | Alpha prefoldin domain |
| βPFD | Alpha prefoldin domain |
| AAA+ | ATPases associated with diverse cellular activities |
| ATM | Ataxia-telangiectasia mutated |
| ATR | ATM- and RAD3-related |
| CCT | Complex containing TCP-1 |
| CS | CHORD domain-containing protein and Sgt1 domain |
| DNA-PKcs | DNA–protein kinase catalytic subunit |
| ECD | Ecdysoneless homolog |
| Hsp70 | Heat shock protein 70 |
| Hsp90 | Heat shock protein 90 |
| mRNP | Messenger ribonucleoprotein |
| mTOR | Mammalian target of rapamycin |

Table 1. (continued)

| | |
|---|---|
| NUFIP1 | Nuclear FMRP interacting protein 1 |
| PAQosome | Particle for arrangement of quaternary structure |
| PDRG1 | p53 and DNA damage regulated 1 |
| PFDL | Prefoldin-like |
| PFDN | Prefoldin complex |
| PIH | PIH1 homology domain |
| Pih1 | Protein interacting with Hsp90 |
| PIH1D1 | PIH1 domain-containing protein 1 |
| PIKK | Phosphatidylinositol-3-kinase-related kinase |
| POLR2E | RNA polymerase II subunit E |
| RNAP | RNA polymerase |
| RNP | Ribonucleoprotein |
| R2TP | Rvb1–Rvb2–Tah1–Pih1 |
| RPAP3 | RNA polymerase II-associated protein 3 |
| RPB5 | RNA polymerase II subunit B5 |
| RUVBL | RuvB-like AAA ATPase |
| SMG-1 | Nonsense-mediated mRNA decay associated phosphatidylinositol-3-kinase-related kinase |
| snoRNP | Small nucleolar ribonucleoprotein |
| snRNP | Small nuclear ribonucleoprotein |
| Tah1 | TPR-containing protein associated with Hsp90 |
| TELO2 | Telomere maintenance 2 |
| TPR | Tetratricopeptide repeat |
| TRRAP | Transformation/transcription domain-associated protein |
| TSC | Tuberous sclerosis complex |
| TTI1 | TELO2 interacting protein 1 |
| TTI2 | TELO2 interacting protein 2 |
| URI1 | Unconventional prefoldin RPB5 interactor 1 |
| UXT | Ubiquitously expressed transcript |
| WDR | WD-40 repeat domain |
| WDR92 | WD-40 repeat domain 92 |
| ZNHIT | Zinc finger HIT-type containing |

complexes related to protein synthesis, cell growth and metabolism, as well as gene expression and genome stability. We believe that additional clients remain to be identified.

While interactions with clients might be direct or indirect, specificity factors (or adaptors) have been shown to participate in the binding of R2TP/PFDL to PIKK proteins (TELO2), box C/D snoRNPs (NUFIP1, ZNHIT3, and ZNHIT6), and U5 snRNP (ZNHIT2 and ECD) (Figure 2), providing a unique mechanism of substrate selection by R2TP/PFDL. In the few cases investigated with recombinant proteins, interaction with clients was shown to occur at least in part through adaptors, with ZNHIT2 being a clear example in the case of the interaction with U5 snRNP [6,8].

Numerous affinity purification experiments performed with R2TP/PFDL, client complexes, and adaptors revealed the transient nature of the interactions between R2TP/PFDL and its client complexes [6,8]. Interaction of R2TP/PFDL with client subunits often increases when their assembly is prevented, indicating that the interaction preferentially occurs with the free subunits [5,8]. Another interesting feature of R2TP/PFDL is its ability to independently interact with several subunits of the client complexes, providing a mechanism to assemble them

together [8,11]. Furthermore, silencing or downregulation of the R2TP/PFDL proteins or adaptors affects the integrity of the client complexes [6,8]. Hence, the results so far fully support a role of R2TP/PFDL in complex formation. Therefore, it can be proposed that R2TP and R2TP/PFDL are *bona fide* chaperones specialized in the assembly of quaternary structures of cellular complexes. To more explicitly reflect the function of this important cellular machinery, we propose to rename the R2TP/PFDL complex as **PAQosome** for **particle for arrangement of quaternary structure**.

## Potential Mechanism of Action

The mechanism of action of the R2TP complex or of the PAQosome has not been elucidated and is the subject of intense investigation; however, the AAA+ ATPases are believed to be the key catalytic components of the complex. A recent study suggested a mechanism whereby yeast Rvb1/2 act as a chaperone by cycling between single and double ring oligomers via their domain II, while holding and remodeling substrates for complex assembly [12]. In this way, Rvb1/2 activity was suggested to be substrate and nucleotide driven [13]. While it is not yet clear how Tah1 and Pih1 might regulate such an oligomerization-based chaperone cycle, recent structure of yeast R2TP provided some interesting clues [14]. In the published structure, the Tah1/Pih1 heterodimer sits within the Rvb1/2 heterohexamer basket and, thus, might be able to regulate the dimerization of the Rvb1/2 ring. The structure provides a rationale of how R2TP would function as a scaffolding platform by bringing together Hsp90 and multiple substrates for complex assembly.

While still speculative, a common feature among the different models is that the Rvb1/2 domain II plays a fundamental role in the function of the complex. These domains are not only responsible for Rvb1/2 dodecamerization, client binding, and association of Pih1/Tah1, but they are also flexible structures that are sensitive to the nucleotide state of the enzymes and, hence, must drive the function of these ATPases.

## Questions and Future Directions

Although a role of the PAQosome in the assembly of protein complexes appears unquestionable, our understanding of the molecular basis of its activity is still at its early stages. Many questions need to be addressed to elucidate the functions of the PAQosome. This will entail cell-based as well as structure-based efforts.

Clarifying the cellular function of the PAQosome will require the identification of additional clients and specificity factors (adaptors) as well as the identification of pathways, molecules, and post-translational modifications regulating PAQosome activity. Better defining the link with other chaperones is also essential. This will involve determining how the PAQosome regulates HSP90 activity and vice versa; whether the PAQosome regulates CCT activity and vice versa; and elucidating potential HSP70 functions with the PAQosome.

Unraveling the mechanism of action is also a key element that needs to be addressed by determining the high-resolution structure of the PAQosome in the presence and absence of substrates, adaptors, chaperones, and nucleotides. It will also require establishing *in vitro* functional assays for the assembly of protein complexes by the PAQosome. This will make it possible to detail the roles of PIH1D1/RPAP3 in client recruitment and in regulating PAQosome activity and will further allow the determination of the role of the specificity factors. Finally, it is at present unclear why certain complexes are particularly dependent on R2TP/PFDL, and, hence, it will be important to define the features shared among PAQosome substrates.

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