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Review

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Chaperone-like activity of the AAA+ proteins Rvb1 and Rvb2 in the assembly of various complexes

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Rvb1 and Rvb2 are highly conserved and essential eukaryotic AAA+ proteins linked to a wide range of cellular processes. AAA+ proteins are ATPases associated with diverse cellular activities and are characterized by the presence of one or more AAA+ domains. These domains have the canonical Walker A and Walker B nucleotide binding and hydrolysis motifs. Rvb1 and Rvb2 have been found to be part of critical cellular complexes: the histone acetyltransferase Tip60 complex, chromatin remodelling complexes Ino80 and SWR-C, and the telomerase complex. In addition, Rvb1 and Rvb2 are components of the R2TP complex that was identified by our group and was determined to be involved in the maturation of box C/D small nucleolar ribonucleoprotein (snoRNP) complexes. Furthermore, the Rvbs have been associated with mitotic spindle assembly, as well as phosphatidylinositol 3-kinase-related protein kinase (PIKK) signalling. This review sheds light on the potential role of the Rvbs as chaperones in the assembly and remodelling of these critical complexes.

1. What are Rvb1 and Rvb2?

Rvb1 and its paralogue Rvb2, with 43 per cent sequence identity and 65 per cent sequence similarity to each other (for the human proteins), belong to the AAA+ (adenosine triphosphases associated with diverse cellular activities) superfamily of ATPases. This class of ATPases is present in all kingdoms of life and is divided into numerous groups, clades and families based on structural and sequence analyses [1–3]. AAA+ proteins usually form hexameric ring structures and are characterized by the presence of the AAA+ module, which contains the highly conserved Walker A and Walker B motifs responsible for nucleotide binding and hydrolysis, respectively [4].

Rvb1 and Rvb2 are known under diverse names such as Pontin/Reptin, TIP49/TIP48, RuvBL1/RuvBL2 and ECP54/ECP51, respectively, reflecting their appearance in many cellular protein complexes and their discovery by unrelated approaches in multiple organisms [5–9]. In this review, we refer to these two proteins as Rvb1 and Rvb2.

2. Discovery and roles of Rvb1 and Rvb2

Rvb1 was originally discovered in 1997 as part of a complex with the TATA-binding protein (TBP) in rat [10]. Rvb1 and Rvb2 were found in complex with the large RNA polymerase II holoenzyme oligomer in 1998 [11], and, subsequently, Rvb2 was identified as an interacting partner of Rvb1 in human cells in 1999 [12]. Rvb1 and Rvb2 share limited sequence similarity (approx. 30%) to the bacterial RuvB helicase [13,14]. RuvB drives the branch migration and resolution of the Holliday junction in complex with RuvA and RuvC during homologous recombination and DNA repair [15]. This sequence similarity suggested that the Rvbs might have helicase activity using ATP binding and hydrolysis, since the deletion of *RVB1* and *RVB2* genes in *Saccharomyces cerevisiae* was complemented by the overexpression of the bacterial RuvAB

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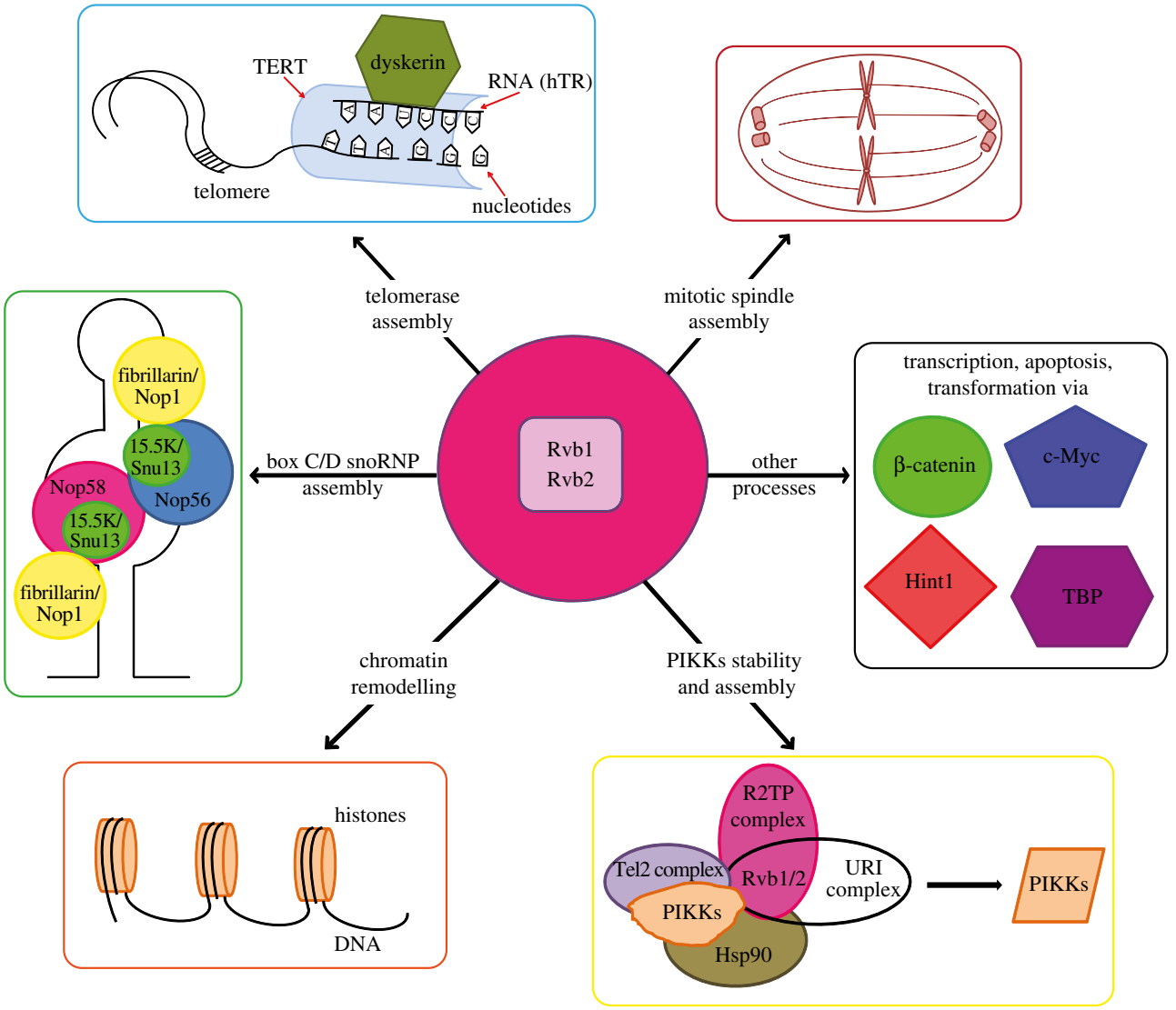

Figure 1. Overview of Rvb1/2 function. Rvb1 and Rvb2 function in the assembly of multiple cellular complexes/processes. They are involved in the assembly of mitotic spindles, telomerase complex, box C/D snoRNPs, chromatin remodelling complexes, and PIKKs. They also exhibit other roles/functions in processes such as transcription, transformation and apoptosis by interacting with factors including β-catenin, c-Myc, Hint1 and TBP. TERT, telomerase reverse transcriptase.

complex [16] and since Rvb1 was found to be associated with the human replication protein (RP)A3 [11]. Indeed, the purified proteins exhibit weak helicase activity [12,17].

The *RVB1* and *RVB2* genes were found to be essential for viability in all model organisms examined so far, including *S. cerevisiase* [11], *Drosophila melanogaster* [8] and *Caenorhabditis elegans* [18], and are speculated to be also essential in mammalian cells. Since their discovery, the Rvbs have been found to be associated with many cellular pathways [19], including chromatin remodelling [9,20–23], transcription regulation [9,24], ribonuleoprotein complex biogenesis [25–29], mitotic assembly [30–32], telomerase complex assembly [33], RNA polymerase II assembly [26,34] and phosphatidylinositol 3-kinase-related protein kinase (PIKK) signalling [29] (figure 1).

3. The structure of Rvb1 and Rvb2

Based on the X-ray structure of human Rvb1 [18], the Rvb sequence can be divided into three domains (figure 2*a*,*b*): (i) an N-terminal αβα subdomain of the AAA+ domain, (ii) a 170 amino acid-insertion domain unique to the Rvbs among the AAA+ proteins which mediates DNA/RNA binding and shows similarity to the ssDNA binding domain of the replication factor replication protein A (RPA), and (iii) an all α subdomain of the AAA+ domain. In the AAA+ domain (figure 2*a*,*b*), the Walker A and Walker B motifs are responsible for ATP binding and hydrolysis, respectively, while sensor I and sensor II motifs sense whether the protein is bound to di- or tri-phosphates. The arginine finger (Arg-finger) of one subunit extends into the ATPase site of the neighbouring subunit and allows coordination of ATP hydrolysis between the subunits in the hexamer [3].

The crystal structure of human Rvb1 has been solved as a hexamer [18] (figure 2*b*), however, the homohexamer was found to be inactive as a helicase and ATPase, suggesting that this might not be the physiologically relevant complex. There is no crystal structure of Rvb2 alone; however, more recently, the crystal structure of the human Rvb1–Rvb2 complex, with truncation of the insertion domain in both proteins, was solved [35] (figure 2*c*). The complex was found to be a dodecamer composed of two hetero-hexameric rings with alternating Rvb1 and Rvb2 monomers [35]. The study showed that the

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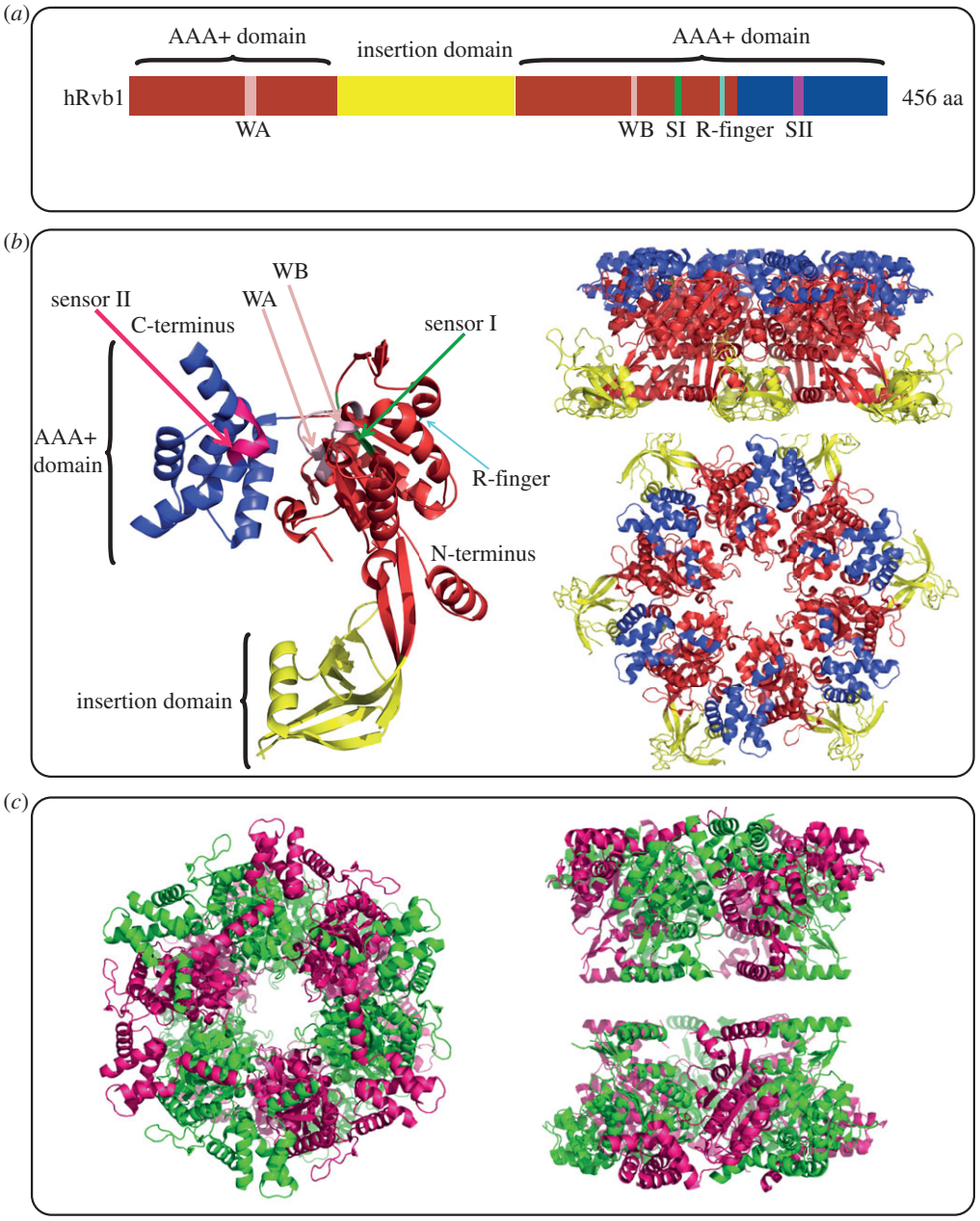

Figure 2. Overview of the Rvb1/2 structure. (*a*) Bar graph of the domain organization of human Rvb1. In red is the N-terminal αβα subdomain of the AAA+ domain, in blue is the C-terminal all α subdomain of the AAA+ domain, and in yellow is the insertion domain. Conserved motifs with the AAA+ domain are also highlighted: WA, Walker A; WB, Walker B; SI, Sensor I; SII, sensor II; R-finger, arginine finger. (*b*) Crystal structure of human Rvb1 monomer on the left-hand side. Side and top view of human Rvb1 hexamer are shown on the right-hand side. The colour scheme used is the same as the one in (*a*). (*c*) Top and side views of the crystal structure of dodecameric human Rvb1/2 complex with truncation in part of Domain II. Human Rvb1 monomers are shown in green and human Rvb2 monomers are shown in pink.

truncated version of the complex exhibits an enhanced ATPase and helicase activity compared with the wild-type (WT) complex, thus, suggesting that the insertion domain functions as a regulator of the activity of the complex.

Using multiple biophysical techniques including analytical ultracentrifugation, size exclusion chromatography, mass spectrometry and electron microscopy, it has been found that human Rvb1 and Rvb2 can form various oligomeric states that are modulated by the insertion domain [36,37]. The oligomeric state of yeast Rvb1 and Rvb2 was also found to be modulated by the presence of a tag at the N-terminus [17,38,39]. These observations seem to indicate that the Rvbs are capable of forming different oligomeric states depending on the complex or cellular process in which they are involved and that other proteins and cofactors might modulate the oligomeric state and, consequently, the activity of the Rvbs.

4. Chaperone-like activity of the Rvbs

Several studies have demonstrated a role of the Rvbs in the assembly of various complexes in different organisms suggesting that they might have a chaperone-like activity. The low abundance of Rvb1 and Rvb2 in eukaryotic cells relative to other components of several complexes which they are part of suggests that the Rvbs are not permanently associated with each complex, therefore providing further support for a general chaperone-like activity of the Rvbs rather than a defined catalytic activity within each complex [40]. In order

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to understand the exact role of the Rvbs in each process/complex, many studies mutated different domains/motifs in the Rvbs and assessed their effects on the activity of the complexes or on the processes being studied. Table 1 summarizes the main reported mutations and their effects as relevant to this review. The chaperone-like activity of the Rvbs in different complexes is further discussed below.

5. Role of Rvb1 and Rvb2 in the assembly of chromatin remodelling complexes

Organisms use DNA as their genetic substance, therefore, DNA-related processes such as transcription, recombination, replication and repair are very critical. The eukaryotic DNA is packaged into chromatin in the nucleus. Nucleosomes form the fundamental repeating units of eukaryotic chromatin. The canonical nucleosome includes about 147 base pairs of DNA wrapped in approximately two superhelical turns around a histone octamer composed of two histone H2A–H2B heterodimers and a histone $(H3–H4)_2$ heterotetramer [49]. Non-canonical nucleosomes have one or more histone variants (e.g. H2A.Z) replacing the canonical histones [49]. The compaction of DNA into a smaller volume is critical for the regulation of the above-mentioned DNA-related processes; however, it also impedes DNA transcription, replication and repair. Several chromatin remodelling complexes modulate these processes by using one of the following mechanisms to facilitate the access of proteins/cofactors to the underlying DNA: (i) using the energy of ATP hydrolysis to slide nucleosomes along the DNA, (ii) adding or removing covalent modifications on the tails of the histones in the nucleosome core or (iii) exchanging canonical histones with histone variants [50,51]. Over the last few years, several studies revealed that Rvb1 and Rvb2 are associated with various chromatin remodelling complexes such as the Ino80 complex in *S. cerevisiae*, *Homo sapiens* and *D. melanogaster* [9,20,21], the SWR-C complex in *S. cerevisiae* [52], and its homologous SRCAP in *H. sapiens* [53–56], and the Tip60 complex in *H. sapiens* and *D. melanogaster* [53,57–59] (figure 1).

(a) The Ino80 complex

The multisubunit Ino80 complex is very well studied and was first purified from yeast by immunoprecipitation [20]. This complex is involved in transcription regulation, replication and repair of DNA double strand breaks by catalysing ATP-dependent mobilization of nucleosomes along the DNA [20,21]. The core subunits of the Ino80 complex are common between yeast and human: the SNF2 family helicase Ino80, which is the catalytic subunit of the complex, Rvb1, Rvb2, Act1, Ino80 subunit (Ies)2 and Ies6 [52], and the actin-related proteins Arp4, Arp5 and Arp8. In addition, the yeast and human Ino80 complexes have their own distinct set of additional subunits. Both yeast and human Ino80 complexes exhibit ATP-dependent nucleosome remodelling activity and DNA and nucleosome-activated ATPase activity [21].

In yeast, considerable overlap was found between genes regulated by Ino80 protein and those regulated by Rvb1 and Rvb2 [9]. The promoters of those genes were found to be associated with the Ino80 protein but not with Rvb1 or Rvb2 [9]. The Ino80 complex has ATPase activity ascribed largely to the Ino80 protein rather than the Rvbs since mutating the ATP-binding site of the Ino80 protein results in significant reduction in the ATPase activity of the complex without affecting the subunit composition of the complex [9]. However, loss of the Rvbs leads to the loss of Arp5 protein from the complex, and, consequently, the loss of the chromatin remodelling activity of the Ino80 complex [45]. The association between Arp5 and the Rvbs requires ATP but not the ATPase activity of the Rvbs [45]. Recently, Chen *et al.* [60] showed that in human Ino80 complex, Rvb1 and Rvb2 together with Arp5, Ies2 and Ies6 associate with an insertion region within the ATPase domain of the Ino80 protein (figure 3*a*).

The Ino80 complex in yeast causes the proximal eviction of nucleosomes surrounding double strand breaks [52]. The Rvb proteins were found to be recruited to the homothallic switching (HO) endonuclease-induced DNA double-strand break along with Arp8, Arp5 and Ino80 protein [52]. This recruitment of the Ino80 complex was dependent upon the phosphorylation of the histone variant H2AX. Deletion of Arp4 and Nhp10 (two subunits of the Ino80 complex) caused a reduction in the recruitment of the complex, including the Rvbs, to the double strand breaks, therefore suggesting that these two proteins are necessary for the recognition of the phosphorylated histones and for the interaction of the Ino80 complex with the double strand break [52]. The exact role of the Rvbs in this recruitment process is yet to be determined. It can be speculated that the Rvbs are required to recruit the rest of the Ino80 subunits to the double strand breaks to form a functional complex.

(b) SWR/SRCAP complex

The Swi/Snf2-related (SWR) complex in yeast, also known as the Snf2-related CREBBP activator protein (SRCAP) complex in mammalian cells, is yet another chromatin remodelling complex that contains both Rvb1 and Rvb2 as integral subunits. Both the SWR and SRCAP complexes were found to remodel chromatin by catalysing the ATP-dependent replacement of H2A–H2B histone dimers in nucleosomes by dimers containing the histone variant Htz1 in yeast or H2AZ in mammalian cells [54,55,61]. This mechanism is essential in a range of cellular processes, such as transcriptional regulation, chromosome segregation, cell cycle progression and DNA damage response. The catalytic subunit of the complex is the Swr1/SRCAP protein, which is an SNF2 helicase. Rvb1, Rvb2, Act1, Arp4, Arp6 and Yaf9/GAS41 are shared subunits between the SWR complex in yeast and the SRCAP complex in mammalian cells [20,52,56]. The SWR/SRCAP complex shares several subunits with the Ino80 complex, namely Act1, Arp4, Rvb1 and Rvb2. In yeast, it was shown that the ATPase domain of the Swr1 protein binds Rvb1, Rvb2, Arp6, Swc2, Swc3 and Swc6 [62] (figure 3*a*), reflecting yet another similarity with the Ino80 complex. The exact function of the Rvbs in the SWR/SRCAP complex remains unexplored. However, given the significant similarity between the SWR complex and the Ino80 complex, it can be speculated that the Rvb proteins perform a role in the assembly of the SWR complex by binding and recruiting a subunit integral for the activity of the complex, just as they recruit Arp5 to the Ino80 complex [45].

(c) Tip60 complex

This complex, which is a histone acetyltransferase (HAT) found in both human and fly cells, remodels chromatin by acetylating histones converting chromatin to euchromatin, which is a

Table 1. Summary of main reported mutations in Rvb1 and Rvb2 and their effect on the respective processes/complexes.

mutated protein: Rvb1 or Rvb2	complex studied	cellular process involved	mutation	effect	organism	reference
Rvb1	interaction with c-Myc	c-Myc-mediated cellular transformation	Walker B mutant D302N	inhibition of c-Myc-mediated cellular transformation but did not affect general growth of cells	rat	Wood *et al*. [7]
Rvb1	interaction with c-Myc	c-Myc-mediated cellular transformation	deletion of Walker A (Δ63–135) and Walker B (Δ290–366) motifs	no effect on the binding to c-Myc	rat	Wood *et al*. [7]
both	—	growth	Walker A and Walker B	incapable of supporting growth	yeast	Jonsson *et al*. [9]
Rvb1	R2TP?	snoRNP biogenesis	Walker A (K81A)	inhibiting snoRNA production	yeast	King *et al*. [41]
Rvb2	interaction with ATF-2	DNA damage repair, apoptosis	Walker B	does not affect interaction with ATF-2 and still represses function of ATF-2	human cell lines	Cho *et al*. [42]
Rvb2	Interaction with β-catenin	expression of endogenous β-catenin/TCF target genes	*Liebeskummer* (*lik*) : 9 bp insertion (FCR a.a.)	enhanced ATPase activity leading to enhanced repression of β-catenin/TCF signalling leading to hyperplastic heart growth	zebrafish	Rottbauer *et al*. [43]
Rvb1	interaction with β-catenin	β-catenin-mediated neoplastic transformation	Walker B mutant D302N	blocked expression of endogenous β-catenin/TCF target genes (e.g. ITF-2 and Axil)	rat	Feng *et al*. [44]
Rvb2	Ino80	chromatin remodelling	Walker B	no effect on recruitment of Arp5 to the Ino80 complex. No effect on chromatin remodelling function of the complex	yeast	Jonsson *et al*. [45]
both	interaction with c-Myc	cell proliferation	deletion of Walker A (Δ70–77 in Rvb1, Δ76–83 in Rvb2) and Walker B (Δ302–306 in Rvb1 and Δ299–303 in Rvb2) motifs	deletion mutants lacking WA or WB motifs did not affect cell division. Therefore not crucial in stimulating cell proliferation	*Xenopus*	Etard *et al*. [46]
both	PcG and TrxG	Hox gene transcription	ATPase domain	abolish normal control of Hox gene expression	*Drosophila*	Diop *et al*. [47]
Rvb1	—	telomerase biogenesis	Walker B D302N	telomerase synthesis could not be rescued by expression of ATPase-deficient Rvb1	human cell lines	Venteicher *et al*. [33]

(*Continued*.)

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Table 1. (*Continued.*)

mutated protein: Rvb1 or Rvb2	complex studied	cellular process involved	mutation	effect	organism	reference
Rvb2	interaction with Influenza virus A polymerase	viral RNA synthesis	Walker B	does not affect interaction with the polymerase and still inhibits its activity	human cell lines	Kakugawa *et al.* [48]
both	—	PIKKs signalling	Walker B	PIKKs expression could not be rescued by the ATPase-deficient Rvbs	human cell lines	Izumi *et al.* [29]
Rvb1	—	PIKKs signalling	Walker B	SMG-1–mediated Upf1 phosphorylation could not be rescued by the expression of Walker B mutant of Rvb1	human cell lines	Izumi *et al.* [29]
both	—	—	truncation of one third of insertion domain	enhanced ATPase and helicase activity of the complex	*in vitro*	Gorynia *et al.* [35]

relaxed, transcriptionally active DNA [59,63]. It has been shown that this complex also acetylates proteins such as the ataxia telangiectasia mutated (ATM) protein kinase after DNA damage, therefore activating ATM [64]. The Tip60 complex is involved in transcription, DNA repair and apoptosis [65]. The catalytic subunit in the complex is Tip60 (Tat interactive protein 60). The complex has Rvb1 and Rvb2 as its integral subunits. The Tip60 complex shares several subunits with the SWR complex and several other subunits with the NuA4 (Nucleosomal Acetyltransferase of H4) complex, which is an acetyltransferase complex found in yeast but that does not contain Rvb1 or Rvb2, suggesting that the Tip60 complex is a fusion of those two complexes [53]. Esa1 in yeast, which is the orthologue of the Tip60 protein in mammals, is the catalytic subunit of the NuA4 complex. Eaf1, a subunit found in the NuA4 complex, is the orthologue of the mammalian p400/domino protein found in the Tip60 complex; however, Eaf1 lacks the ATPase domain found in p400/domino protein [53,57]. The absence of Rvb1 and Rvb2 in the NuA4 complex may be because of the absence of the ATPase domain in Eaf1 since Rvb1 and Rvb2 were shown to interact with the ATPase domain of p400/domino [53] (figure 3*a*), similarly to the way they interact with the ATPase domain of the Ino80 protein.

As mentioned above, Tip60 is involved in DNA damage repair. DNA damage causes histone variant H2AX to be phosphorylated by ATM and ATR protein kinases. The phospho-H2AX acts as a marker that recruits other proteins to the sites of DNA damage to amplify the damage signal and repair the damage [52,63]. HAT activity of Tip60 is required to acetylate H4 before the phospho-H2AX can be remodelled and dephosphorylated in DNA damage response [63]. It has been shown that depletion of either Rvb1 or Tip60 causes an increase in the phosphorylated H2AX and that the Rvbs are required for the HAT activity of the Tip60 complex, suggesting that Rvb1 is required for the assembly of the Tip60 complex [63].

The role of Rvb1 is also linked to apoptosis through Tip60. Tip60 is required for the acetylation of p53, and the acetylation of p53 is required for its binding to promoters of proapoptotic genes [66]. In another example, Feng *et al* [44] showed that the stable expression of the Walker B mutant of Rvb1 blocked the expression of endogenous β-catenin/T-cell factor (TCF) target genes, which is because of inhibition of histone acetylation of β-catenin/TCF target gene sequences, thus suggesting that Rvb1 exerts its effect through Tip60 [44]. Also, Rvb1, along with Tip60, binds to and acetylates histones at the promoter of KAI1, which is a metastasis suppressor gene, resulting in the induction of the expression of KAI1 [67].

6. Role of Rvb1 and Rvb2 in box C/D snoRNP biogenesis

In an attempt to identify the interactors of yeast Hsp90, which is a ubiquitous molecular chaperone that is essential in many signalling pathways, our group conducted systematic genome-wide screens and found Rvb1 and Rvb2 to be components of a complex interacting with Hsp90 that we termed the R2TP complex [68]. In yeast, this complex consists of two Hsp90 interactors, which we identified and termed Tah1 (tetratricopeptide repeat (TPR)-containing protein associated with Hsp90) and Pih1 (protein interacting with

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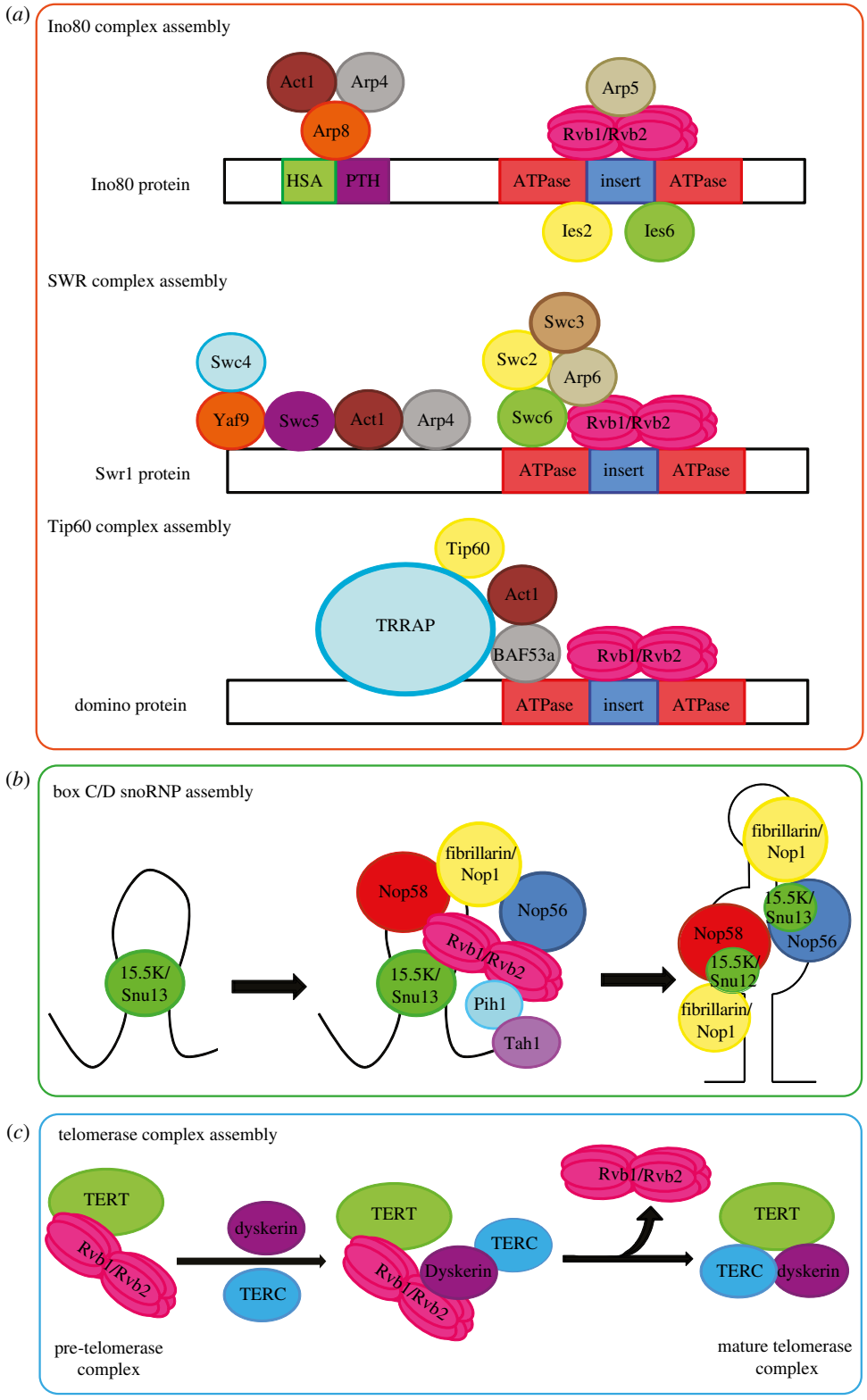

Figure 3. Chaperone-like activities of the Rvbs. (*a*) Rvb1 and Rvb2 assemble the Ino80 complex by recruiting Arp5 to the Ino80 protein. Rvb1 and Rvb2 have a possible role in the assembly of the SWR complex by binding and recruiting subunits integral for the activity of the complex. Finally, Rvb1 and Rvb2 interact with the ATPase domain of domino/p400, and help in the assembly of the Tip60 complex (TRRAP, transformation/transcription domain-associated protein). (*b*) Rvb1 and Rvb2 function in the assembly of box C/D snoRNP by bridging interactions between 15.5K/Snu13 and the other core proteins. (*c*) Rvb1 and Rvb2 bring together TERT, dyskerin and TERC (telomerase RNA component) and remodel the pre-telomerase complex into a mature TERT–TERC–dyskerin complex.

Hsp90), and the two AAA+ helicases Rvb1 and Rvb2 [68], hence the name R2TP. Tah1, which was uncharacterized at the time and whose structure we solved recently [69], consists of two TPR motifs and a C-terminal helix. Tah1 was found to bind to the MEEVD peptide corresponding to the C-terminus of Hsp90, while the C-terminus of Tah1 binds to the C-terminus of Pih1 [69]. Pih1, also uncharacterized at the time, is a 40 kDa protein which was found to be unstable on its own, and stable upon binding to the C-terminus of Tah1 [69].

The R2TP complex is highly conserved in eukaryotes. In humans, R2TP contains Rvb1, Rvb2, RPAP3 (protein equivalent to Tah1 although not similar) and PIH1D1 (Pih1 orthologue) [26]. The R2TP complex has been implicated in small nucleolar ribonucleoprotein (snoRNP) assembly and

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pre-ribosomal RNA processing in human and yeast cells [26,27]. The complex also plays essential roles in apoptosis, PIKK signalling [29] and RNA polymerase II assembly [70].

snoRNP complexes are made up of either box C/D or box H/ACA small nucleolar RNAs (snoRNAs) complexed with proteins. snoRNPs are involved in cleavage and modification of small nuclear RNA (snRNA), ribosomal RNA (rRNA) and tRNAs [71]. Box C/D snoRNPs catalyse ribose 2′-O methylation of pre-ribosomal RNA (pre-rRNA), while box H/ACA snoRNPs mediate pseudo-uridylation of pre-rRNA [28]. Mature box C/D snoRNAs in eukaryotes are associated with four common core proteins: 15.5K (Snu13 in yeast), NOP56, NOP58 and the methyltransferase fibrillarin (Nop1 in yeast) [28]. The core box C/D proteins bind a conserved sequence termed the box C/D motif that folds into a stem-internal loop-stem structure known as a k-turn (figure 3*b*). 15.5K, an RNA binding protein, binds directly to the k-turn to recruit the other core proteins [72–74]. The assembly of the complete complex is essential for the nucleolar localization of the complex [74]. Several proteins are required for this assembly, including the R2TP complex, as well as, NUFIP, TAF9 and BCD1 [28,41,75,76] (figure 3*b*). It has been shown that Rvb1 and Rvb2 weakly interact with NOP56, NOP58 and fibrillarin, and that the presence of ATP stimulates the interaction of Rvb1 and Rvb2 with 15.5K [28]. Rvb1 and Rvb2 appear to bridge the interaction between 15.5K and both NOP56 and NOP58 proteins [28]. In both yeast [27,41] and mammalian cells [28], depletion of the Rvbs results in the mislocalization of the snoRNP core proteins. The data to date indicate that the Rvb proteins play an important role in the assembly and remodelling of the snoRNP complex during biogenesis (figure 3*b*) mainly as components of the R2TP complex.

7. Role of Rvb1 and Rvb2 in PIKK signalling

Recent studies revealed that Rvb1 and Rvb2 are common regulators of all phosphatidylinositol 3-kinase-related protein kinase (PIKK) members [77]. PIKKs are serine–threonine protein kinases with catalytic domains homologous to those of phosphatidylinositol 3-kinases. PIKKs regulate DNA damage responses, nutrient-dependent signalling, and nonsense-mediated mRNA decay (NMD) [77]. The PIKK family includes DNA-PKcs (DNA-dependent protein kinase catalytic subunit), ATM and ATR (ATM- and Rad3-related), which are collectively responsible for signalling the presence of DNA damage [77]. They phosphorylate proteins that have roles in regulation of cell cycle progression, DNA repair, apoptosis and cellular senescence [77]. The PIKK family also includes SMG-1 (suppressor with morphological effect on genitalia 1), mTOR (mammalian target of rapamycin) and TRRAP (transformation/transcription domain-associated protein) in mammals [77]. SMG-1 is an essential factor of NMD and TRRAP regulates transcription as a subunit of HAT complexes [78]. SMG-1 and TRRAP are also involved in DNA damage signalling and repair [78]. A multiprotein complex called SMG1C, which is composed of SMG-1, SMG-8 and SMG-9, is essential for NMD. SMG1C detects and degrades mRNAs to prevent the production of potentially harmful premature proteins [29]. mTOR regulates nutrient-dependent signalling.

Knockdown of human Rvb1 or Rvb2 has been shown to lead to decreased phosphorylation of direct downstream effectors of ATM, ATR, mTOR and SMG-1, and also to decreased abundance of mRNA and proteins for ATM, ATR, DNA-PKcs, TRRAP and mTOR but not the abundance of other kinases [29]. WT Rvb1 or Rvb2 were able to rescue the reduced PIKK abundance, however, ATPase-deficient mutants failed to rescue the reduced abundance, indicating that the ATPase activities of both Rvb1 and Rvb2 are required to control the abundance of PIKKs [29]. It was also revealed that human Rvb1 and Rvb2 are required for SMG-1-mediated Upf1 phosphorylation, which occurs on a spliced mRNP in the cytoplasm, and that the phosphorylation was dependent on the ATPase activity of Rvb1. This phosphorylation is induced by remodelling of the mRNA surveillance complex that involves first the formation of the SURF complex, which is composed of SMG1, UPF1, eRF1 and eRF3, on a ribosome recognizing premature termination codon(s) and then the formation of the decay-inducing (DECID) complex on an mRNP. Immunoprecipitation experiments suggested that the Rvb1/2 complex associates with SURF playing a role in the remodelling of the surveillance complex and, thus, in forming a DECID complex [29].

In addition, human Rvb1/2, as part of the R2TP complex, plays a role in the assembly and stabilization of the PIKKs. This stability and assembly is achieved when the R2TP-Hsp90/Prefoldin-like complex interacts with PIKKs via the Tel2 complex (also known as the TTT complex), which is composed of Tel2, Tti1 and Tti2 [78]. A recent study in yeast linked the Tel2 complex and Asa1p to PIKKs [79].

8. Role of Rvb1 and Rvb2 in telomerase complex assembly

Telomeres are repetitive nucleotide sequences located at the ends of chromosomes, capping and protecting them from degradation and recombinogenic activities. They are un-replicated and lost during cell division owing to the ‘end replication problem’ exhibited during DNA replication, and are replenished by the telomerase [80]. The end replication problem is a problem the DNA polymerase runs into because the leading strand in the double-stranded DNA can be replicated to the very end, but the lagging strand cannot. The polymerase needs RNA primers to replicate the lagging strand DNA; however, use of the RNA primer is not possible at the end of the DNA because there is nothing for the primer to bind to, therefore, the last section of the lagging strand cannot be synthesized. Thus, after several cycles of replication, the DNA would continue to get smaller.

Telomerase is a multisubunit RNP complex that adds DNA repeats to telomeres. The complex is composed of the catalytic subunit TERT (telomerase reverse transcriptase), TERC (telomerase RNA component) and the TERC-binding protein dyskerin [33]. In humans, Rvb1 and Rvb2 were identified as subunits of the telomerase complex, and they were found to be required for telomerase assembly/biogenesis through maintaining the telomerase RNA stability [33]. It was demonstrated that Rvb1 directly interacts with TERT, recruiting Rvb2 and bridging its interaction to the TERT complex. It was also shown that Rvb1 and Rvb2 interact with dyskerin [33]. Depletion of Rvb1 and Rvb2 caused a loss of TERC and dyskerin from the complex suggesting that

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dyskerin bridges the interaction between the Rvb proteins and TERC [33]. The Walker B mutant of Rvb1 could not rescue TERC and dyskerin loss from the complex, thus indicating that Rvb1 and Rvb2 are essential for telomerase activity and for TERC and dyskerin accumulation through a mechanism that requires ATPase activity [33]. Rvb proteins seem to help bring together TERT, dyskerin and TERC and remodel the TERT–Rvb1–Rvb2 complex into a mature TERT–TERC–dyskerin complex [33] (figure 3*c*).

In addition to their role in the assembly of the telomerase complex, Rvb1 and Rvb2 seem to be also involved in the transcription of TERT [81]. Knocking down Rvb1 or its partner Rvb2 using siRNA in gastric and cervical cancer cells led to significant decreases in TERT mRNA. In addition, human Rvb2 depletion resulted in a significant decrease in the activity of TERT promoter that is dependent on c-MYC [81]. Therefore, TERT transcription requires the constitutive expression of Rvb2 and its cooperation with c-MYC.

In yeast, the Rvbs are also subunits of what is called the ASTRA complex [79,82]. ASTRA (ASsembly of Tel, Rvb, and Atm-like kinase) complex is composed of Tra1 (TRRAP homolog), Rvb1, Rvb2, Tel2 (telomere binding protein), Tti1p, Tti2p and Asa1p (a WD-repeat-containing protein). The ASTRA complex is poorly studied, but it is proposed to play a role in telomeric maintenance and its components (Tti1p, Tti2p, Tel2 and Asa1p) have been shown to be linked to PIKKs as mentioned previously. The role of the Rvbs within this complex is not yet characterized.

9. Role of Rvb1 and Rvb2 in mitotic spindle assembly

Several studies reported the involvement of Rvb1 and Rvb2 in mitosis. Human Rvb1 was found to copurify with tubulin isolated from U937 cells [30]. Furthermore, human Rvb1 was found to colocalize with tubulin at the centrosome and at the mitotic spindle in addition to being present in the nucleus. Using an *in vitro* tubulin assembly assay, it was demonstrated that Rvb1 is involved in the formation of microtubules [30]. Subsequently, another study showed that Rvb2 associates with the centrosome and the mitotic spindle [31]. However, it was demonstrated that, unlike Rvb1, Rvb2 localizes to the midzone during telophase and to the midbody during cytokinesis [31]. In 2008, Ducat *et al*. [32] demonstrated that depletion of Rvb1 using siRNA causes a defect in spindle assembly in *Drosophila* and mammalian cell lines. The same result was observed when depleting Rvb1 in *Xenopus* egg extracts. Moreover, Rvb1 and Rvb2 were found to interact with the γ-tubulin ring complex in *Xenopus*, which is involved in nucleating spindle formation, suggesting that both Rvb proteins are involved in mitotic spindle assembly.

10. Role of Rvb1 and Rvb2 in cancer

In mammalian cells, Rvb1 and Rvb2, separately and together, were found to have a crucial role in pathways linked closely to cancer. Several studies have shown that both Rvb1 and Rvb2 are overexpressed in 80 per cent of colon cancer specimens. Rvb2 is found to be overexpressed in human hepatocellular carcinoma cells, while Rvb1 transcript levels are found to be increased in non-small cell lung cancer [83]. The transcription of both genes is deregulated in several cancers such as liver, bladder and melanoma. In addition, it has been demonstrated that decreasing the expression of Rvb1 or Rvb2 results in reduced tumor cell growth and increased apoptosis *in vitro* and that decreasing Rvb2 expression results in growth arrest of established tumours in xenograft experiments in mice [83].

The roles of the Rvbs associated with modulating cellular transformation, signalling, apoptosis and response to DNA damage is mediated through their interaction with a multitude of proteins such as the tumor suppressor protein Hint1 and the transcription factors β-catenin, c-Myc, E2F (only Rvb1) and ATF2 (only Rvb2) [6–9].

11. Role of Rvb1 and Rvb2 in transcription regulation

Rvb1 and Rvb2 can function together but in several cases have also been shown to function independently and to exhibit antagonistic effects on the regulation of transcription of several target genes. Rvb1 and Rvb2 interact with β-catenin, which is a major player in Wnt signalling that affects TCF-mediated transcription [8]. In the nucleus, stable unphosphorylated β-catenin binds to the TCF family of transcription factors and increases the expression of downstream genes (e.g. *c-Myc*, *ITF-2* and *Cox-2*) [44]. Rvb1 and Rvb2 have opposing effects on β-catenin-TCF transcriptional activity. Rvb1 increases the transcriptional activation of target genes, while Rvb2 represses the β-catenin/TCF transactivation complex and thus decreases the transcription of downstream genes [8]. The Walker B mutant of Rvb1 was found to block β-catenin-mediated transcription of TCF-dependent genes owing to inhibition of acetylation of histones near β-catenin target gene sequences suggesting that Rvb1/Tip60 mediates the regulation of this transcription [44]. On the other hand, Rvb2 represses gene activation mediated by β-catenin and TCF through its interaction with histone deacetylase HDAC1 and 2, and corepressor TLE (transducin-like enhancer) [8]. In another example, Rvb1/Tip60 are recruited on the promoter of the KAI1 (a metastasis suppressor which inhibits metastasis by promoting cell adhesion) gene as a co-activator complex, while Rvb2/β-catenin act as a co-repressor of the transcription which recruits HDAC1 as well [84]. In addition, Hint1 (histidine triad nucleotide-binding protein 1), which acts as a co-regulator of β-catenin-TCF-mediated transcription, was shown to bind to the insertion domain in Rvb1 and Rvb2 [85]. It was demonstrated that Hint1 prevents formation of hetero and homo complexes of the Rvbs, but not the interaction between the Rvb proteins with β-catenin. Hint1 was found to be a regulator of the Rvbs/Wnt-catenin signalling pathway since its overexpression was found to modulate Rvbs/β-catenin regulated genes.

Rvb1 and Rvb2 were found to bind to and regulate the function of the transcription factor c-Myc [7] (table 1). c-Myc, which is involved in oncogenic transformation, apoptosis and stimulation of cell proliferation, contains two conserved regions: Myc homology box I (MBI) and MBII, with the latter being the region where both Rvb1 and Rvb2 bind. The Walker B mutant form of Rvb1 was found to inhibit c-Myc oncogenic activity but did not inhibit cellular growth indicating that Rvb1 is essential for c-Myc-mediated oncogenic transformation [7].

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12. Concluding remarks

Rvb1 and Rvb2 are involved in various cellular complexes and processes in different organisms. They exhibit different roles and functions specific to the processes in which they are involved. Besides being ATPases that provide energy for several processes and helicases with potential DNA/RNA unwinding activity, many studies have shown that the Rvbs seem to act as chaperones. They have been found to recruit proteins/DNA/RNA to their respective complexes and to remodel these complexes by bridging the interactions between the different components within the complex. Hence, we propose that the Rvbs are potential chaperones for the assembly and maturation of protein–protein and protein–DNA/RNA complexes. However, further studies need to be conducted to determine the exact role of the Rvbs in the assembly of these complexes.

Note added in proof

While this review was in preparation for publication, the crystal structure of human Rvb2 with truncation in part of Domain II was published by Petukhov *et al*. [86] and the cryo-electron microscopy structures of human double-ring Rvb1-Rvb2 complexes were published by López-Perrote *et al*. [87]. In addition, the role of human Rvbs (through the R2TP complex) in H/ACA RNP biogenesis was established by Machado-Pinilla *et al*. [88]

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