MINIREVIEW / MINISYNTHÈSE

Rvb1–Rvb2: essential ATP-dependent helicases for critical complexes[1]

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Abstract: Rvb1 and Rvb2 are highly conserved, essential AAA+ helicases found in a wide range of eukaryotes. The versatility of these helicases and their central role in the biology of the cell is evident from their involvement in a wide array of critical cellular complexes. Rvb1 and Rvb2 are components of the chromatin-remodeling complexes INO80, Swr-C, and BAF. They are also members of the histone acetyltransferase Tip60 complex, and the recently identified R2TP complex present in *Saccharomyces cerevisiae* and *Homo sapiens*; a complex that is involved in small nucleolar ribonucleoprotein (snoRNP) assembly. Furthermore, in humans, Rvb1 and Rvb2 have been identified in the URI prefoldin-like complex. In *Drosophila*, the Polycomb Repressive complex 1 contains Rvb2, but not Rvb1, and the Brahma complex contains Rvb1 and not Rvb2. Both of these complexes are involved in the regulation of growth and development genes in *Drosophila*. Rvbs are therefore crucial factors in various cellular processes. Their importance in chromatin remodeling, transcription regulation, DNA damage repair, telomerase assembly, mitotic spindle formation, and snoRNP biogenesis is discussed in this review.

Key words: Rvb1, Rvb2, helicase, chromatin remodeling, snoRNP assembly.

Résumé : Rvb1 et Rbv2 sont des hélicases à domaine AAA+ hautement conservées, trouvées chez un large spectre d'eucaryotes. La versatilité de ces hélicases et leur rôle central dans la biologie de la cellule est prouvé par leur implication dans un large éventail de complexes cellulaires importants. Rvb1 et Rvb2 sont des composantes des complexes de remodelage de la chromatine INO80, Swr-C et BAF. Elles sont aussi membres du complexe de l'acétyltransférase d'histone Tip60 et du complexe R2TP récemment identifié chez *Saccharomyces cerevisiae* et *Homo sapiens* et impliqué dans l'assemblage des petites ribonucléoprotéines nucléolaires (snoRPN). De plus, chez l'humain, Rvb1 et Rvb2 ont été identifiées au sein du complexe URI de type préfoldine. Chez la drosophile, le complexe répresseur Polycomb contient Rvb2 mais pas Rvb1, et le complexe Brahma contient Rvb1 mais pas Rvb2. Ces deux complexes sont impliqués dans la régulation des gènes liés à la croissance et le développement chez la drosophile. Donc, les Rvb constituent des facteurs cruciaux dans plusieurs processus cellulaires. Leur importance dans le remodelage de la chromatine, la régulation de la transcription, la réparation des dommages à l'ADN, l'assemblage de la télomérase, la formation du fuseau mitotique et la biogenèse des snoRNP est discutée dans cet article de synthèse.

Mots-clés : Rvb1, Rvb2, hélicase, remodelage de la chromatine, assemblage des snoRNP.

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Discovery and naming of Rvb1 and Rvb2

The ATPases associated with a variety of cellular activities (AAA+) superfamily is made up of a large number of proteins involved in various cellular activities. Rvb1 and Rvb2 are 2 essential AAA+ helicases present in eukaryotes. Rvb1 was first identified in 1997 as part of a complex with TATA box binding protein (TBP) and named TBP-interacting protein 49 or Tip49 (Kanemaki et al. 1997). Since then, Rvb1 has been given such names as Tip49a, RuvBL1, Pontin52, TAP54α, Tih1p, p50, NMP238, and ECP54 (Kane-

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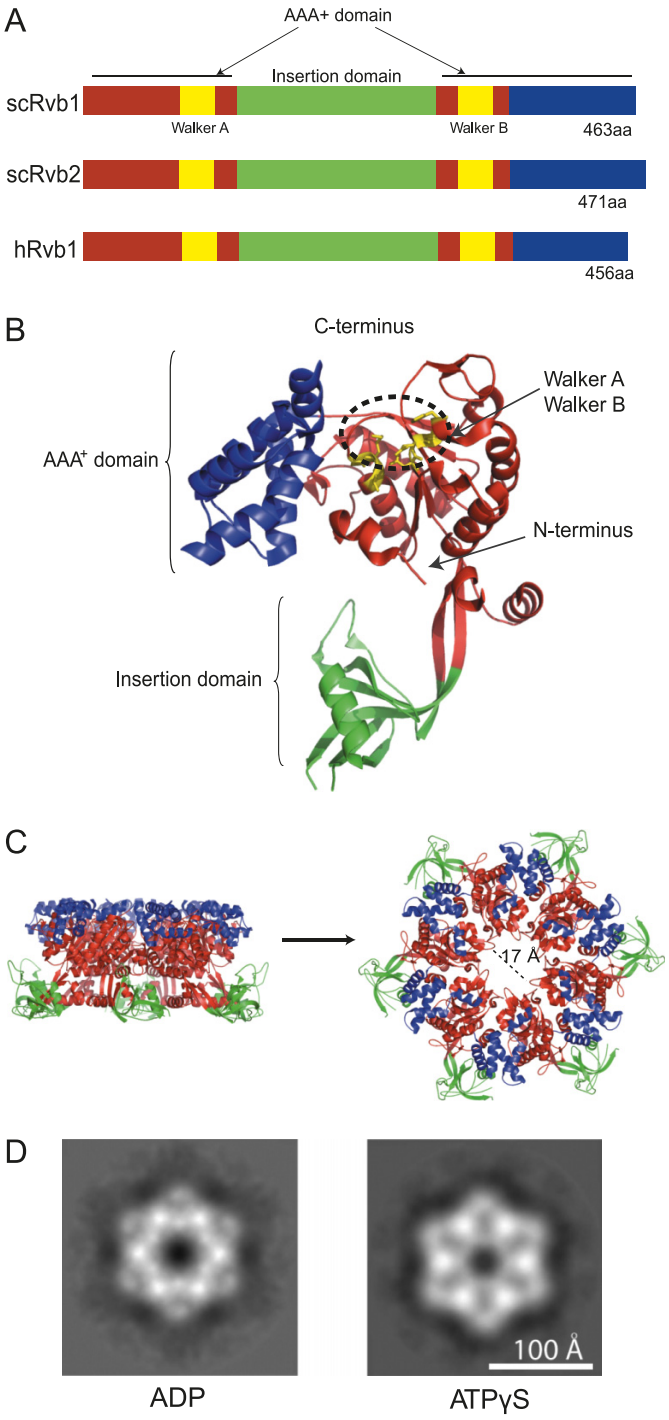


Fig. 1. Rvb1 and Rvb2 architecture. The full-colour version of this Figure is available from http://bcb.nrc.ca. (A) Domain organization of yeast and human Rvb1 and Rvb2. The AAA+ domain consists of the αβα subdomain (red) and the all-α subdomain (blue). The Walker A and Walker B ATP-binding and hydrolyzing motifs are also shown (yellow). The insertion domain (green) is so named because of its absence in the RuvB helicase in *Escherichia coli*. (B, C). The X-ray structures of the monomeric and hexameric human Rvb1 are shown as a ribbon representation. The structure is from the PDB file 2C90 (Matias et al. 2006) and was drawn using Pymol (http://www.pymol.org/). The colour scheme used is the same as that in A. (D) Negative-staining electron microscopy projection structures of the yeast Rvb1–Rvb2 complex in the ADP and ATPγS states (Gribun et al. 2008).

maki et al. 1997; Bauer et al. 1998; Holzmann et al. 1998; Qiu et al. 1998; Makino et al. 1999; Salzer et al. 1999; Ikura et al. 2000; Lim et al. 2000; Shen et al. 2000; Fuchs et al. 2001). Rvb2, a paralogue of Rvb1, was identified as part of a complex with Rvb1 that took part in c-Myc mediated cell transformation in HeLa cells (Wood et al. 2000). Alternate names for Rvb2 are Tip48, Tip49b, RuvBL2, Reptin52, TAP54β, Tih2p, p47, and ECP51 (Qiu et al. 1998; Gohshi et al. 1999; Kanemaki et al. 1999; Salzer et al. 1999; Bauer et al. 2000; Ikura et al. 2000; Lim et al. 2000; Wood et al. 2000; Fuchs et al. 2001). In this review, we will refer to them as Rvb1 and Rvb2.

Oligomeric state of Rvb1–Rvb2

Both Rvb1 and Rvb2 contain the canonical αβα subdomain and the all-α subdomain that together form the AAA+ domain (Fig. 1A,B). Members of the AAA+ superfamily are ATPases that usually self assemble into larger ring-like oligomeric complexes mediated by interactions of the AAA+ domains (Iyer et al. 2004; Ammelburg et al. 2006). The Walker A and Walker B motifs, along with a strictly conserved arginine from the neighboring monomer, form part of the ATPase pocket. Structural studies have shown that Rvb1 and Rvb2 together can form a complex of 2 stacked hexameric rings in vitro (Puri et al. 2007; Torreira et al. 2008). Furthermore, the crystal structure of human Rvb1 alone has been solved as a hexamer (Fig. 1C) (Matias et al. 2006). However, using various methods, our laboratory recently demonstrated that recombinant yeast Rvb1 and Rvb2, when mixed together, form a single heterohexameric ring (Gribun et al. 2008) that undergoes nucleotide-dependent conformational changes (Fig. 1D; Cheung et al. 2010). Hence, the exact oligomeric state of Rvb1 and Rvb2 within the cellular context remains under debate. However, we favor the argument that the heterohexameric complex is the physiologically relevant Rvb1–Rvb2 complex, at least in yeast, because we have established that this complex has both ATPase and weak helicase activities (Gribun et al. 2008), whereas the double hexameric complex has dubious ATPase activity and no demonstrated helicase activity. However, it should also be emphasized that Rvb1 and Rvb2 might also act independently of one another.

Activities of Rvb1 and Rvb2

The AAA+ domain of Rvb1 and Rvb2 are weakly homologous to the bacterial RuvB helicase, involved in branch migration and Holliday junction resolution (Parsons et al. 1992; Hishida et al. 2004). RuvB was shown to be active in unwinding DNA in the 5′→3′ direction (Tsaneva et al. 1993), but literature has presented controversial results with regards to the ATPase and DNA unwinding activity of Rvb1 and Rvb2. Some of these results indicate that rat Rvb1 alone and human Rvb2 alone are active ATPases and helicases (Kanemaki et al. 1999; Makino et al. 1999). Ikura et al.

(2000) showed that recombinant yeast Rvb1 and Rvb2 together exhibit enhanced ATPase activity compared with Rvb1 or Rvb2 alone. This is in agreement with the enhanced helicase activity that we observed for the Rvb1–Rvb2 complex compared with Rvb1 or Rvb2 alone (Gribun et al. 2008). In addition, our group showed that the presence of DNA with 3′ or 5′ overhangs greatly enhanced the ATPase activity of the Rvb1–Rvb2 complex (Gribun et al. 2008). However, the Rvb1–Rvb2 complex from *Homo sapiens* was shown to have ATPase activity but lacked helicase activity (Puri et al. 2007). Other groups were unable to detect ATPase or helicase activities for human Rvb1 (Qiu et al. 1998; Matias et al. 2006). The requirement for cofactors or substrates could explain the apparent lack of activity observed by some groups. It was demonstrated that recombinant human Rvb1 and Rvb2 exhibit lower ATPase activity than when part of the Tip60 complex, suggesting that other factors may be involved in modulating the ATP activity of the Rvbs (Ikura et al. 2000; Fuchs et al. 2001). It was also proposed that binding of ADP locked the helicase in a rigid conformation, thus rendering it ATPase inactive (Matias et al. 2006).

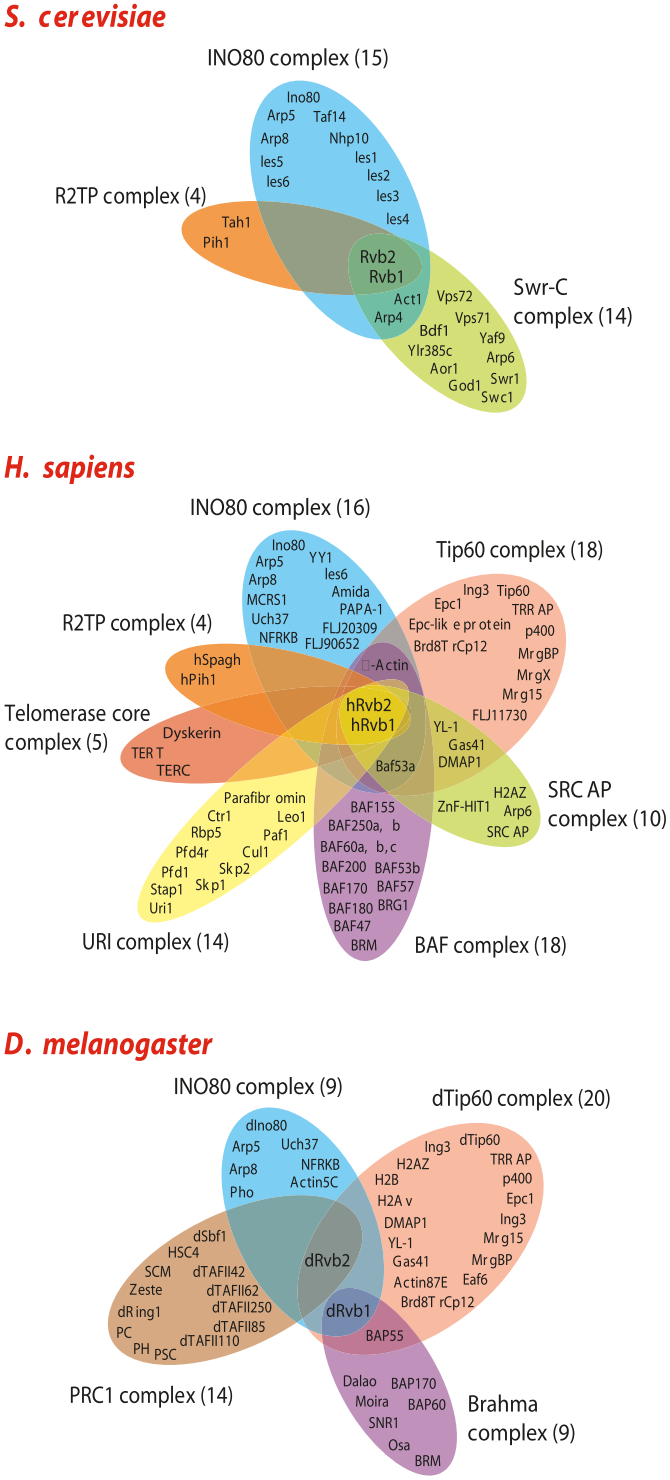
Results from investigations of Rvb function in vivo have been more consistent. Rvb1 and Rvb2 are each essential genes in yeast: deletion of either gene results in non-viability (Qiu et al. 1998; Kanemaki et al. 1999; Radovic et al. 2007). Rvb1 or Rvb2 knockdown in human cell lines results in premature senescence and cell cycle arrest (Chan et al. 2005). Overexpression of either Rvb1 or Rvb2 is toxic in yeast (Sopko et al. 2006), and diploid strains that are heterozygous for either Rvb1 or Rvb2 are haploinsufficient in DNA damage repair (Radovic et al. 2007).

Thus, it is not surprising that the Rvbs play key roles in a number of cellular processes. They are essential components of the INO80 and Swr-C chromatin-remodeling complexes and the Tip60 histone acetyltransferase complex. They have been shown to interact with various transcription factors that are necessary for cell cycle progression such as c-Myc and β-catenin. They are critical factors in DNA damage repair, telomerase core complex assembly, and are involved in regulating mitotic spindle formation. The Rvbs are also significant players in the biogenesis of small nucleolar ribonucleoprotein (snoRNP). This review will describe their involvement in these processes.

Involvement in DNA remodeling

The cell has devised several methods for decompacting tightly wound DNA for exposure to important factors and complexes such as those involved in transcription, DNA replication, recombination, and repair. One such mechanism is by mobilizing nucleosomes to modulate the spread of tightly packed heterochromatin DNA. The INO80 complex (Fig. 2) is a large hetero-subunit complex that utilizes this mechanism (Shen et al. 2000). Ino80 protein, first identified through its involvement in transcriptional regulation of the inositol biosynthesis pathway (Ebbert et al. 1999), is a DEAD/H box protein that contains helicase-type motifs and was established as an indispensable catalytic component of the INO80 complex (Shen et al. 2000; Jónsson et al. 2004). Components of the INO80 complex were identified by immunoprecipitation of FLAG-tagged Ino80 from yeast lysates

Fig. 2. Rvb1–Rvb2-containing protein complexes in budding yeast (*Saccharomyces cerevesiae*), human (*Homo sapiens*), and fly (*Drosophila*). Equivalent complexes in the different organisms are presented in the same shade.



and subsequent analysis of co-immunoprecipitated proteins by mass spectrometry, thus resulting in the identification of Rvb1 and Rvb2 (Shen et al. 2000). Ohdate et al. (2003) demonstrated that the genes affected by reduction in Rvb2 levels substantially overlapped with those affected in an *ino80* knockout strain. Purified yeast INO80 complexes

were shown to exhibit DNA unwinding activity in an ATP-dependent manner, but this activity was abolished when Ino80 was replaced with the Ino80-K737A ATPase-deficient mutant (Shen et al. 2000). It was suggested that the helicase activity of the Rvbs are functionally coupled to the Ino80 ATPase (Shen et al. 2000). Furthermore, Jónsson et al. (2004) showed that depletion of Rvb2 rendered the INO80 complex inactive in nucleosome mobility experiments and that this depletion resulted in a loss of the critical Actin-related protein 5, Arp5, from the complex. Although Rvb1 was not tested, the proposition is that both Rvb1 and Rvb2 are essential in the recruitment of Arp5 to form a functional INO80 complex (Jónsson et al. 2004). The mammalian INO80 complex (Fig. 2) is not as well characterized, but was shown to bear similarities to the yeast complex (Jin et al. 2005).

Another mode of chromatin remodeling involves the replacement of histones with histone variants. The nucleosome consists of a histone octamer made up of 2 molecules each of H2A, H2B, H3, and H4, with approximately two turns of DNA (147 bp) wrapped around the octamer (Sarma and Reinberg 2005). Core histones are exchanged with histone variants, each of which are associated with specific DNA-related processes (Malik and Henikoff 2003; Sarma and Reinberg 2005). The replacement of H2A with variant H2AX has been observed to correlate with homologous recombination and transcription (Stargell et al. 1993; Malik and Henikoff 2003). It was proposed that the Swr-C complex employs such a mechanism for chromatin remodeling in yeast (Krogan et al. 2003). Specifically, Krogran et al. (2003) demonstrated that in the absence of Swr-C components, fewer H2AX histone variants were recruited to DNA. Rvb1 and Rvb2 were identified in the yeast Swr-C complex and in the homologous SRCAP (Swi2–Snf2-related CPB activator protein) complex in mammals (Krogan et al. 2003; Kobor et al. 2004; Mizuguchi et al. 2004; Cai et al. 2005). However, the exact role of the Rvbs in these complexes remains unclear.

Interestingly, the Rvbs are not present in the yeast Swi–Snf complex, although they have been identified in the human BAF complex, the mammalian Swi–Snf homolog (Fig. 2). In *Drosophila melanogaster*, the homologous complex is called Brahma and contains Rvb1 (Pontin), but lacks Rvb2 (Reptin), whereas the Polycomb repressive 1 chromatin-remodeling complex (PRC1 complex) contains Rvb2 and lacks Rvb1 (Tamkun 1995; Reisman et al. 2009). These 2 complexes are crucial for cell differentiation and organogenesis in *Drosophila* (Tamkun 1995). Various groups have shown that certain protein complexes contain only one of either Rvb1 or Rvb2 (Cho et al. 2001; Ghaemmaghami et al. 2003; Etard et al. 2005; Diop et al. 2008). Diop et al. (2008) demonstrated that the Rvb1-containing Brahma complex and Rvb2-containing PRC1 complex function antagonistically in the regulation of the *Drosophila* homeotic Hox genes. The fact that Rvb1 or Rvb2 are individually found in the aforementioned complexes seems to imply that the helicases also have independent functions (Shao et al. 1999; Conaway and Conaway 2009; Reisman et al. 2009).

Histone acetyltransferases (HATs) alter chromatin structure by acetylating target histones, resulting in DNA relaxation and exposure to proteins located in the vicinity. The human and fly Tip60 (Tat-interactive protein 60) complexes are HATs that are homologous to the yeast NuA4 HAT complex (Doyon et al. 2004; Kusch et al. 2004). The Rvbs are not components of the NuA4 complex, but have been shown to be essential factors in the mammalian Tip60 complex (Ikura et al. 2000; Jha et al. 2008). The human Tip60 complex shares several components with the INO80 and SRCAP complex (Fig. 2), suggesting that the mammalian Tip60 complex arose from a fusion of the NuA4 complex with the INO80 or Swr-C complex (Doyon and Côté 2004).

The Tip60 complex plays critical roles in DNA damage repair because acetylation of histone H2A or variant H2AX is a prerequisite for efficient double-strand break repair (Gospodinov et al. 2009). The complex is targeted to phosphorylated H2AX, a marker for double-stranded breaks, and acetylates the histone variant, resulting in efficient DNA repair (Ikura et al. 2000; Doyon et al. 2004; Jha et al. 2008). It was proposed that human Rvb1 is required for DNA damage repair, as it is required for histone acetyltranserase activity of the Tip60 complex (Jha et al. 2008). In addition, Gospodinov et al. (2009) demonstrated that RNA silencing of either Rvb1 or Rvb2 causes a reduction of Rad51, a critical component of the DNA damage response, at sites of DNA damage. Furthermore, depletion of TRRAP or expression of a Tip60 HAT mutant results in a decline of Rad51 at nuclear foci (Murr et al. 2006; Ikura et al. 2007). Although it is tempting to implicate the Rvbs in DNA damage repair through the Tip60 complex, it cannot be ruled out that they are involved in the DNA damage response via other complexes.

The URI complex has been proposed to regulate genes involved in the nutrient-sensitive TOR1 (target of rapamycin) kinase pathway (Gstaiger et al. 2003; Yart et al. 2005; Parusel et al. 2006; Deplazes et al. 2009). Uri1(unconventional prefoldin RPB5 interactor), is thought to function as a scaffolding protein bringing together other prefoldins (Yart et al. 2005). In HeLa cells, Uri1 forms a complex with Rvb1, Rvb2, the prefoldin STAP1, and RPB5, which is a shared subunit of the 3 RNA polymerases (Gstaiger et al. 2003). Uri1 is involved in the nutrient stress response since Uri1 knockout in *S. cerevisiae* affects expression of amino acid biosynthesis genes (Gstaiger et al. 2003). In *Caenorhabditis elegans*, it was shown that Uri1 is involved in germ line cell proliferation and DNA stability (Kanemaki et al. 1997; Parusel et al. 2006). The exact role of Rvb1 and Rvb2 in the Uri1 complex has yet to be elucidated.

Rvbs interact with transcription factors

As mentioned earlier, Rvb1 was first identified by affinity purification in rat liver nuclear extracts as a TATA-box binding protein (TBP) interactor (Kanemaki et al. 1997). Shortly thereafter, the interaction between the Rvbs and TBP was confirmed in *Drosophila*, yeast, and humans (Bauer et al. 1998; Makino et al. 1998; Ohdate et al. 2003). Various studies have implicated Rvb1 and Rvb2 as integral players in the transcriptional processes. It was demonstrated in conditional *rvb* mutants that Rvb1 and Rvb2 are involved in the transcription of over 5% of yeast genes, many of which are directly involved in cell cycle regulation (Lim et al. 2000; Wood et al. 2000; Jónsson et al. 2001).

In addition to TBP, the Rvbs have also been found to interact with various other transcription factors, such as c-

Myc, β-catenin, and E2F, all of which are critical in regulating cell growth, proliferation, and apoptosis. The downstream effects of this interaction are often drastic. *Drosophila* with a loss of function *rvb1* allele coupled with a mutant *d-myc* allele exhibit severe eye defects: small, irregular, and rough shaped eyes with reduced ommatidia (Bellosta et al. 2005). Flies that only carry the mutant *d-myc* allele show slight reduction in body size, whereas those that are only heterozygous for the *rvb1* allele are morphologically indistinguishable from wildtype flies (Bellosta et al. 2005). This demonstrates that *rvb1* and *d-myc* show a strong genetic interaction (Bellosta et al. 2005). In *Xenopus*, knockdown of either Rvb1 or Rvb2 results in embryonic lethality (Etard et al. 2005) as expected for these essential proteins. It was proposed that lethality was an effect of derepression of the transcription repressor complex c-Myc–Miz1 (Etard et al. 2005). Miz1 is a transcription factor that, when complexed with c-Myc, inhibits transcription of Miz1 promoter genes. Etard et al. (2005) showed that *Xenopus* embryos knocked down for Rvb1 or Rvb2 could be rescued by the overexpression of c-Myc or inhibition of Miz-1, indicating that Rvb1 and Rvb2 may act as co-repressors of the c-Myc–Miz-1 pathway. In early rat embryo fibroblasts, overexpression of both Rvb1 and a constitutively active form of c-Myc is necessary for cellular transformation, as evident by foci formation (Wood et al. 2000; Feng et al. 2003). Rvb1 was also found to be critical for β-catenin/TCF-mediated cell transformation. Feng et al. (2003) observed that an ATPase inactive mutant of Rvb1 expressed in a human colon cancer cell line deregulated of β-catenin resulted in reduced cellular transformation. Rvb2 was not tested in these studies.

Interestingly, there is also evidence that Rvb1 and Rvb2 function antagonistically in the same pathway (Bauer et al. 2000; Rottbauer et al. 2002; Kim et al. 2005). Overexpression of Armadillo, the *Drosophila* homolog of β-catenin, causes bristles to grow in the posterior wing blade (Bauer et al. 2000). Flies that had one mutant allele of Rvb1 result in a suppressed bristle phenotype, whereas flies that lack 1 allele of Rvb2 exhibit an enhanced bristle phenotype (Bauer et al. 2000). This is in agreement with the findings presented by Rottbauer et al. (2002), who showed that Rvb1 and Rvb2 in Zebrafish function antagonistically during heart development. Zebrafish that are homozygous for a mutant of Rvb2 (called *lik*), which renders the protein with intrinsically higher ATPase activity in a DNA-independent manner in comparison with wild-type Rvb2, exhibited cardiac hyperplasia during embryogenesis (Rottbauer et al. 2002). Heterozygotes for *lik* do not exhibit the hyperplasic phenotype, but upon decrease in β-catenin protein levels, the *lik* phenotype became apparent in 75% of embryos (Rottbauer et al. 2002). In contrast, reduction of Rvb1 in wild type embryos phenocopies the morphology of homozygous *lik* mutants (Rottbauer et al. 2002). This suggests that Rvb1 and Rvb2 function as antagonistic regulators in β-catenin signaling.

It remains unclear how the Rvbs are involved in c-Myc-mediated pathways. They play a role in the c-Myc–Miz1 transcription repressor complex, but also function in the c-Myc pathway independent of Miz1. The Rvbs were shown by co-immunoprecipitation to interact with c-Myc in *Drosophila* and in H293T cells (Wood et al. 2000; Bellosta et al. 2005). It was proposed that the Rvbs are cofactors in c-Myc-mediated transcription since they are recruited to the c-Myc target promoter *NUC* (for nucleolin) (Frank et al. 2003). In addition, Tip60 and TRRAP are also recruited to the *NUC* promoter (Frank et al. 2003). These results corroborate with those observed in E2F1 interaction studies. E2F1 directly binds to 3 components of the Tip60 HAT complex: Tip60, TRRAP, and Rvb1 (McMahon et al. 1998; Dugan et al. 2002; Taubert et al. 2004)—all of which, in addition to Rvb2 and p400, were found to bind E2F1 target promoters in an E2F1-dependent manner (Taubert et al. 2004). Recently, it was found that the expression of the tumor suppressor KAI1 in prostate cells was inducible by phorbol 12-myristate 13-acetate (PMA) (Rowe et al. 2008). KAI1 is a member of the tetraspanin family of proteins normally involved in cell adhesion, migration, and signaling and is a potent inhibitor of secondary tumor progression (Malik et al. 2009). Further investigation showed that PMA induction increased acetylation of histones 3 and 4 and recruitment of Rvb1 and Tip60 to the NFκB-p50 promoter (Rowe et al. 2008). These results are in agreement with those published by Kim et al. (2005), who demonstrated that in nonmetastatic prostate cancer cells, knockdown of Tip60 results in decreased histone acetylation and KAI1 expression.

These results suggest that the Rvbs function as adaptor proteins in which the interaction with certain transcription factors leads to targeting of the Tip60 HAT complex to the respective promoter, subsequently leading to histone acetylation and gene expression. Other Rvb-containing complexes are not excluded from this proposed mechanism, as it was found that the SRCAP complex in mammals is essential for c-Myc mediated cellular transformation (Park et al. 2002). In addition, various other transcription factors have been reported to interact with the Rvbs (Cho et al. 2001; Updike and Mango 2007), indicating that the Rvbs are involved in directly regulating gene expression.

Involvement of Rvb1–Rvb2 in snoRNP biogenesis and assembly

The biogenesis of the small nucleolar ribonucleoprotein complex (snoRNP) is a highly orchestrated process that coordinates the transcription of the snoRNA with the assembly and restructuring of the core proteins interacting with the snoRNA. The maturation process involves a number of proteins that specialize in recruitment of other snoRNP proteins, endo- and exoribonucleolytic activities, helicase activities, splicing, and RNA polymerase activity. Only after the proper formation of the mature snoRNP particle can the particle participate in the modification and processing of pre-ribosomal RNA in the nucleolus. snoRNPs can be classified into 3 families: the box C/D snoRNP, responsible for 2′-O-methylation of the ribose on the target nucleotide, the box H/ACA snoRNP, responsible for pseudo-uridylation of the target uridine, and the MRP snoRNP that is thought to act like a ribozyme and directly cleaves pre-rRNA (Lygerou et al. 1996; Newman et al. 2000). Of these 3 snoRNPs, the association of the box C/D snoRNP with Rvb1 and Rvb2 has been best characterized thus far (Watkins et al. 1998; Newman et al. 2000; King et al. 2001; Watkins et al. 2002; Watkins et al. 2004; McKeegan et al. 2007; Boulon et al. 2008; Zhao et al. 2008).

Most mammalian snoRNAs are intron encoded and require a splicing-dependent mechanism to generate the free snoRNA (Richard and Kiss 2006). In yeast, the majority of snoRNAs are encoded on independent genes and rely on splicing-independent processing (Maxwell and Fournier 1995). Since free RNA is rapidly degraded in the cell by 3′ and 5′ exoribonucleases (Terns and Terns 2002; Richard and Kiss 2006), the assembly of snoRNP-associated proteins on the snoRNA is thought to occur co-transcriptionally. The core box C/D snoRNP proteins are nucleolar protein 1 (Nop1), Nop56, Nop58, and small nuclear ribonucleoprotein 13 (snu13). Nop1, or fibrillarin in mammalian cells, is the 2′-O-methyltransferase responsible for methylation of the 2′ oxygen on the target ribose moiety of the pre-rRNA (Tran et al. 2004). Nop58 binds to the box C/D motif of the snoRNA and is responsible for proper box C/D snoRNA accumulation (Lafontaine and Tollervey 2000; Tran et al. 2004). Nop56 binds the box C′/D′ motif and is required for proper pre-rRNA processing (Tran et al. 2004). Snu13 is an RNA binding protein that is required for the recruitment of the other core box C/D proteins (Watkins et al. 2002). The exact order of core protein assembly is still controversial, but it is generally accepted that Snu13 must first bind to the box C/D motif before the other core proteins are recruited (Fig. 3) (Watkins et al. 2000; Cahill et al. 2002; Granneman et al. 2002; Szewczak et al. 2002; Watkins et al. 2002).

Several lines of evidence have suggested that the Rvbs are involved in the early stages of snoRNP biogenesis. First, Rvb1 and Rvb2 are localized to the nucleoplasm, where premature snoRNPs are being formed, and are excluded from the nucleolus, the site of fully matured snoRNPs (King et al. 2001; Watkins et al. 2004). Second, sucrose gradient sedimentation of HeLa cell nuclear extracts reveal that Rvb1 and Rvb2 associate with premature forms of U3 snoRNPs present in the nucleoplasm (Watkins et al. 2004); they do not associate with nucleolar U3 snoRNPs, which are structurally different, fully matured snoRNPs (Watkins et al. 2004). Third, Pih1 (also called Nop17), a Nop58 interacting protein, forms a tight complex with Rvb1 and Rvb2 (Gonzales et al. 2005; Zhao et al. 2005). In yeast, Pih1 is required for the nucleolar localization of the core snoRNP proteins and Pih1 deletion results in accumulation of the 35S pre-rRNA (Gonzales et al. 2005; Zhao et al. 2008). These results implicate Pih1 in pre-rRNA processing through its involvement in snoRNP biogenesis. Our group discovered that Pih1 forms a tight complex with Rvb1, Rvb2, and Tah1 (a small tetratricopeptide repeat (TPR) protein) forming the R2TP complex (Zhao et al. 2005). Prior studies had implicated Pih1 and the Rvbs in snoRNP biogenesis so this prompted us to investigate the role of the R2TP complex in pre-snoRNP assembly.

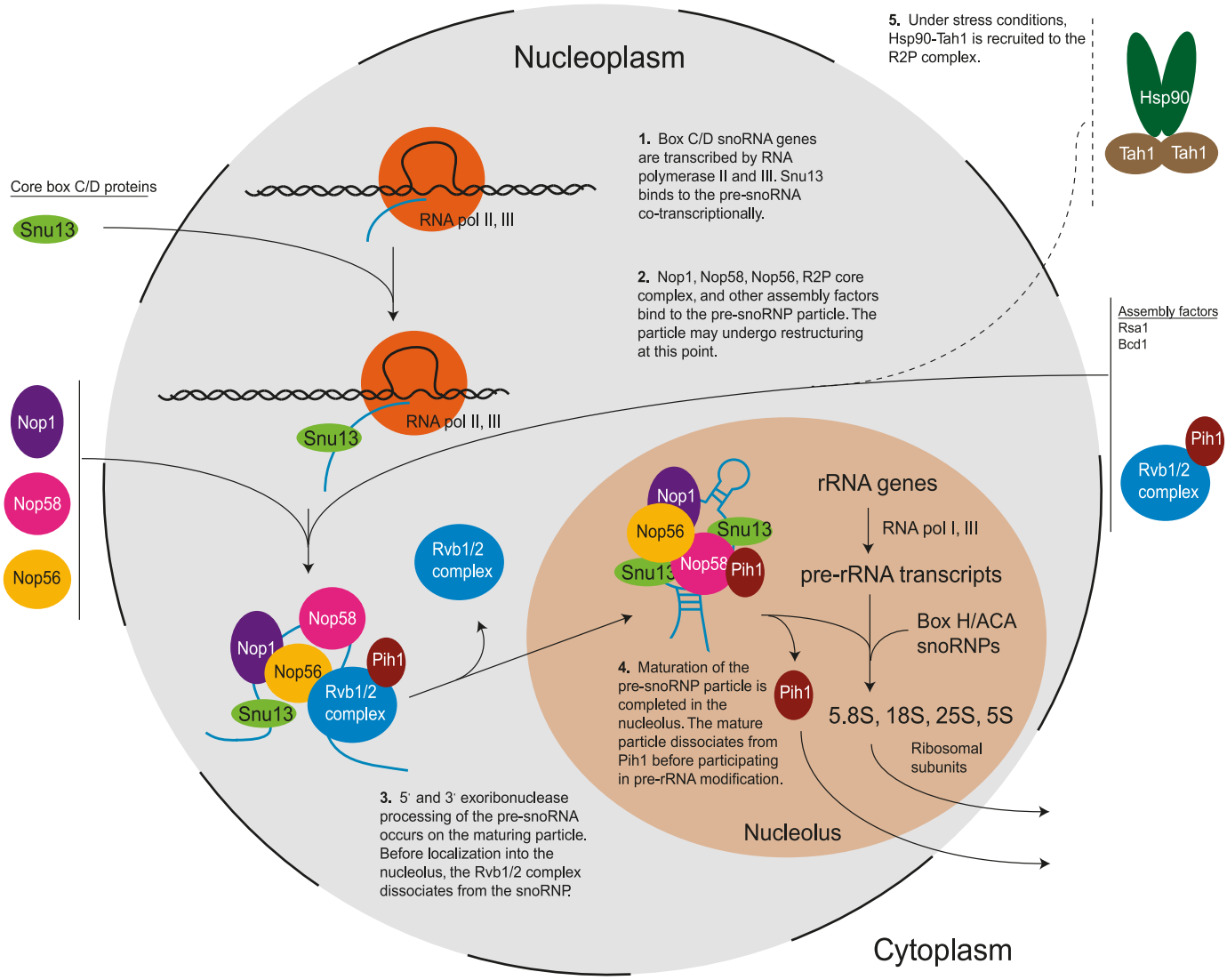
Pih1 and Tah1 were originally identified in a yeast 2-hybrid screen as interactors with the ATP-dependent molecular chaperone Hsp90 (Zhao et al. 2005). Our group observed that Pih1 is a highly unstable protein, tending to form aggregates in vitro, but could be disaggregated in the presence of Hsp90, Tah1, and ATP as observed by atomic force microscopy, light scattering, and size exclusion chromatography (Zhao et al. 2008). Furthermore, yeast cells that were rapidly depleted of Tah1 exhibited a decrease in Pih1 levels as well, suggesting that Pih1 is unstable within the cell and is rapidly degraded. Co-chaperones of Hsp90 commonly recruit the chaperone to client substrate proteins (Young et al. 2001). Thus, it is likely that Hsp90–Tah1 stabilizes Pih1 in vivo (Zhao et al. 2008). But what is the role of the R2TP complex and Hsp90 in snoRNP biogenesis? Using a yeast strain having reduced Hsp90 levels at permissive temperatures, it was demonstrated that in these cells the Nop58–Nop56–Nop1–Snu13 core complex is not properly formed, and that the cells had reduced box C/D snoRNA levels and accumulated 35S pre-rRNAs (Zhao et al. 2008). Pih1 knockout cells exhibited similar results. Accordingly, the reduction or elimination of individual R2TP components also results in the reduction of these box C/D snoRNAs (Zhao et al. 2008).

Interestingly, deletion of Tah1 or depletion of Hsp90 affects snoRNA accumulation especially under stress conditions such as during stationary phase. Deletion of Pih1 or depletion of Rvb1 or Rvb2 affect snoRNA levels under all growth conditions. This suggests that during growth, the R2P core complex is sufficient for function in snoRNP biogenesis, but under a stress-induced environment when proteins are more susceptible to degradation, Hsp90–Tah1 is recruited to the core complex to stabilize Pih1. Consequently, Pih1 can then modulate the interaction between the Rvbs and the snoRNP.

Orthologous components of yeast R2TP were identified in humans (Jeronimo et al. 2007; Te et al. 2007). They are also found to form an R2TP complex that plays a similar role in snoRNP biogenesis (Boulon et al. 2008). Boulon et al. (2008) demonstrated that inhibition of human Hsp90 with geldanamycin, an anti-fungicide that binds to the ATPase pocket of Hsp90, results in the decrease of the U3 and U4 snoRNAs as well as in the reduction of hNop58, and 15.5K (human homolog of Snu13) protein levels. In their study, Boulon et al. also identified 2 novel snoRNP assembly factors: human Nufip and its yeast homolog Rsa1. Nufip, an RNA binding protein, and Rsa1, a ribosome assembly protein, function as adaptors that tether 15.5K and Snu13, respectively, to the other box C/D core proteins. However, Nufip associates with only the nucleoplasmic fraction of snoRNPs and is absent from mature snoRNP particles. This novel assembly factor also associates with Hsp90 and the human homologs of Rvb1 and Rvb2. Taken together, these observations provide further evidence that the R2TP complex and Hsp90 function in the early stages of snoRNP biogenesis as important assembly factors, and suggest that the function of R2TP is conserved from yeast to humans.

A working model of R2TP function in box C/D snoRNP biogenesis is illustrated in Fig. 3. For the sake of simplicity, only the splicing-independent pathway for snoRNP biogenesis is shown. Detailed reviews of snoRNP biogenesis can be found elsewhere (Filipowicz and Pogacić 2002; Kiss 2006; Richard and Kiss 2006). There are several possible roles the R2TP complex might play. After Snu13 binding to the snoRNA transcript, R2TP is localized to the premature snoRNP particle, along with Rsa1 and other assembly factors in the nucleoplasm (Yang et al. 2000; Jones et al. 2001; Verheggen et al. 2001; McKeegan et al. 2007; Boulon et al. 2008). Rvb1 and Rvb2 were found to be linked to Nop58 via Pih1, suggesting that localization of the R2TP complex to the snoRNP particle is also through binding to Nop58 (Watkins et al. 2002; Gonzales et al. 2005). In addi-

Fig. 3. A working model for R2TP function in box C/D snoRNP biogenesis in *S. cerevisiae*. The pre-box C/D snoRNA gene is transcribed by RNA polymerase II or III in the nucleoplasm. Snu13 is proposed to bind first to the newly formed transcript. The other core box C/D proteins, Nop1, Nop58, and Nop56 along with the R2P core complex and other assembly factors then assemble onto the newly formed Snu13-snoRNP particle. The R2P core complex likely assembles during Nop58 assembly. Binding of the core proteins and assembly factors probably occurs co-transcriptionally, since free RNA is subject to rapid degradation. After the core proteins and assembly factors bind to the premature snoRNP particle, 3′ and 5′ exoribonucleases process the pre-snoRNA ends. Restructuring of the pre-snoRNP particle may begin in the nucleoplasm and continue in the nucleolus. Before entry into the nucleolus, the Rvb1–Rvb2 complex dissociates. It is possible that the Rvb1–Rvb2 complex remains bound to the nucleolar snoRNP particle, but this interaction is likely only transient. After maturation of the pre-snoRNP particle, Pih1 dissociates. Under conditions of stress, depicted by dotted lines, the molecular chaperone Hsp90, together with its cofactor Tah1, is recruited to the R2P core complex to stabilize Pih1.



tion, localization of the core box C/D proteins requires the presence of Pih1 (Gonzales et al. 2005), implicating R2TP as an assembly and (or) recruitment factor complex.

After recruitment of the core box C/D proteins, processing of the pre-snoRNA is thought to occur by exo- and endoribonucleases, like the yeast Rnt1 endoribonuclease (Giorgi et al. 2001). This is likely followed by restructuring events that convert the pre-snoRNP particle into the mature particle. Given that the Rvbs are ATP-dependent helicases, it is possible that the R2TP complex functions as a restructuring factor complex that unwinds mispaired RNA bases or displaces improperly bound core proteins. Such an RNAchaperone role has been attributed to Sen1p, a putative RNA helicase involved in maturation of the 3′ termini of the snR13 snoRNA (Rasmussen and Culbertson 1998). In addition, the R2TP complex may behave as a stabilizing factor by simply binding the snoRNP. It is possible that the R2TP complex fulfills all these roles by coordinating the restructuring, stabilization, and recruitment functions through interactions with other snoRNP biogenesis factors.

Rvb1–Rvb2 play an essential role in telomerase core complex assembly

The Rvbs were recently implicated in the assembly of the

telomerase core complex. The telomerase core complex is a ribonucleoprotein particle that consists of 3 essential components: TERT, the telomerase reverse transcriptase; dyskerin, a core box H/ACA RNA-binding protein; and TERC, the telomerase RNA component (Cohen et al. 2007). Purification of TERT-complexes from human cells led to the identification of hRvb1 and hRvb2. Venteicher et al. (2008) demonstrated that depletion of hRvb1 and hRvb2 results in the decline of dyskerin and TERC from the core complex. In addition, Rvb1 and Rvb2 each individually interact with TERT and dyskerin in a TERC-independent manner (Venteicher et al. 2008). In fact, the TERT–Rvb1–Rvb2 complex was recognized as a stable complex, suggesting that the Rvbs bridge TERT to the dyskerin–TERC particle, and thus leading to the formation of the TERT–dyskerin–TERC core telomerase complex (Venteicher et al. 2008). It is possible that the Rvbs may function as remodeling factors for the maturation of the telomerase core complex.

Involvement in mitotic spindle assembly

The nucleation of microtubules at the centrosomes and the growth of these microtubules to form spindles are important events that occur during mitosis. Various studies have implicated Rvb1 and Rvb2 in these processes. In human cells, it was demonstrated that both Rvb1 and Rvb2 localize to spindles and spindle poles (Gartner et al. 2003; Sigala et al. 2005). Reduction of Rvb1 protein levels in HeLa cells results in spindle defective phenotypes such as splayed spindle poles, elongated spindles, reduction of centrosome numbers to one or none, displacement of centrosomes from spindles, and dimmed microtubule staining (Ducat et al. 2008). In addition, cell death is more frequent in Rvb1 knockdown cells, with many exhibiting partially aligned chromosomes and mitotic arrest before initiating apoptosis (Ducat et al. 2008). Reduction in Rvb2 levels enhances this phenotype but was not observed for cells solely knocked down for Rvb2. It was demonstrated in *Xenopus* that both Rvb1 and Rvb2 interact with the γ-tubulin ring complex, which is involved in nucleating spindle formation at centrosomes, suggesting that they may both be directly involved in mitotic spindle assembly (Ducat et al. 2008). Spindle defects are accompanied by prolonged mitotic arrest that could be due to unresolved DNA damage or imbalanced transcription of apoptosis factors, suggesting that the Rvbs could indirectly affect spindle formation through their interaction with chromatin remodeling complexes (Ducat et al. 2008). Hence, it remains unclear the exact function of Rvb1 and Rvb2 in mitotic spindle assembly.

Rvbs in relation to human pathologies

The Rvbs are extensively involved in DNA-associated processes as they are found to interact with a wide range of transcription factors as described above. It is, therefore, not surprising that they have been implicated in the progression of human diseases, especially in carcinogenesis (Carlson et al. 2003; Kim et al. 2007; Lauscher et al. 2007; Rousseau et al. 2007; Yu et al. 2008). In a study of human colon cancer cells, expression of cyclooxygenase-2, a protein known to be crucial in the progression of colon carcinogenesis, was found to be associated with the overexpression of Rvb1 (Carlson et al. 2003). Two independent groups reported the overexpression of Rvb1 in patient colon carcinoma samples (Carlson et al. 2003; Lauscher et al. 2007). In all cases showing Rvb1 upregulation, more β-catenin was found localized in the nucleus. Although it remains unclear how Rvb1 is involved in colon cell carcinogenesis, these results, coupled with molecular data that Rvb1 is necessary for β-catenin/TCF-mediated cell transformation (Feng et al. 2003), suggest that Rvb1 is important in the progression of colorectal cancer through the β-catenin pathway (Lauscher et al. 2007).

Although Rvb2 was not analyzed in the above investigation, other studies in human hepatocellular carcinoma cells have implicated Rvb2 in carcinogenesis. Of 96 cases of hepatocellular carcinoma, 72 were confirmed to overexpress Rvb2 mRNA, and were correlated with poor prognosis, independent of other prognosis variables (Rousseau et al. 2007). It was demonstrated that overexpression of Rvb2 in the HuH7 human hepatocellular carcinoma cell line reduces apoptosis when treated with the apoptotic stimulus, C2 ceramide. Rvb2 overexpression also enhances tumorigenicity as observed in mice injected with these cells (Rousseau et al. 2007). Importantly, it was demonstrated that silencing of Rvb2 transcripts results in reduced tumor cell growth and increased apoptosis (Rousseau et al. 2007). The mechanisms inducing the overexpression of Rvbs in tumors are not fully understood but might be tumor-type specific.

Post-translational modifications of the Rvbs could affect cell cycle regulation and promote cellular transformation. For example, SUMOylation of Rvb1 was shown to increase proliferation and growth of prostate cancer cells (Kim et al. 2007). SUMOylated Rvb1 is necessary for expression of androgen-receptor target genes, suggesting that modification of Rvb1 with SUMO moieties is one way for the cell to regulate growth and proliferation (Kim et al. 2007). A more comprehensive analysis of Rvb1 and Rvb2 in relation to carcinogenesis is presented by Huber et al. (Huber et al. 2008).

Conclusion

Although the Rvbs are essential components of a wide range of complexes, it is expected that their role and their mode of function will be very similar in all those complexes. Their activities and targets, nevertheless, are expected to be modulated by the proteins that they interact with in those complexes. Our group was able to show both ATPase and helicase activities for both Rvb1 and Rvb2 as well as for the Rvb1–Rvb2 complex for the first time. Future studies will concentrate on determining the molecular basis of these activities to unveil the structural details of the mechanism of function of these essential helicases. Furthermore, our understanding of the biological roles of Rvb1–Rvb2 is still at its infancy, and it is expected that these proteins will be major players in the future research endeavors of many groups.

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