**Short Conceptual Overview**

Yoshito Kakihara* and Makio Saeki

# The R2TP chaperone complex: its involvement in snoRNP assembly and tumorigenesis

**Abstract**: R2TP was originally identified in yeast *Saccharomyces cerevisiae* as Hsp90 interacting complex, and is composed of four different proteins: Rvb1, Rvb2, Tah1, and Pih1. This complex is well-conserved in eukaryotes, and is involved in many cellular processes such as snoRNP biogenesis, RNA polymerase assembly, PIKK signaling, and apoptosis. An increasing number of research related to R2TP suggests a linkage of its function with tumorigenesis. In this review, we provide an overview of several recent studies on R2TP that are related to cell proliferation and carcinogenesis, and propose a possible role of R2TP in tumorigenesis through regulating snoRNA/snoRNP biogenesis.

**Keywords:** box C/D snoRNP; box H/ACA snoRNP; R2TP; snoRNP biogenesis; tumorigenesis.

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## Introduction

R2TP is composed of four different proteins: Rvb1, Rvb2, Pih1, and Tah1. It was initially identified by high-throughput screening of Hsp90 interacting proteins (1). R2TP is conserved from yeast to human, and each component has diverse names because of different naming conventions, as shown in Table 1 (2–5). Rvb1 and Rvb2 belong to the AAA+ (ATPases associated with various cellular activities) superfamily of proteins (5). Pih1 (Protein interacting with Hsp90), which belongs to Pih1 family of proteins, directly interacts with Rvb1-Rvb2 complex (Rvbs) and Tah1 (TPR [tetratricopeptide repeat-containing protein] associated with Hsp90) (1, 3, 6). In yeast, Pih1 is an unstable protein and prone to aggregate *in vitro*, however, the Pih1 aggregates are dissociated by Hsp90 in an ATP-dependent manner, and its disaggregation activity of Hsp90 is enhanced by Tah1 (6). Tah1 is suggested to function as a co-chaperone of Hsp90 in disaggregating Pih1 (6). As shown in Figure 1, it has been determined that Rvbs are involved in many different cellular processes such as transcriptional regulation, DNA repair, telomerase assembly, and mitotic spindle assembly (7–12), in contrast, when in complex with Tah1 and Pih1, R2TP's cellular function is limited to apoptosis, PIKK signaling, snoRNP biogenesis, and RNA polymerase II assembly (Figure 1) (2–4, 12, 13). Also, R2TP (or at least Rvbs) is found in ASTRA (ASsembly of Tel, Rvb and Atm-like kinase) complex which is suggested to be involved in telomere maintenance and TORC1 signaling in yeast (14, 15). It has been demonstrated that Pih1 functions as an adaptor protein. In yeast, Pih1 targets R2TP to Nop58 (a core factor in box C/D snoRNP) (16). In mammals, PIH1D1 targets R2TP to dyskerin (yeast Cbf5 homologue, a core factor in box H/ACA snoRNP) (17), Rpb1 (a core subunit in RNA polymerase II) (18), and Tel2 [a core subunit in TTT (Tel2-Tti1-Tti2) complex] (19, 20).

In this review, we will focus on describing the R2TP function in snoRNP biogenesis. snoRNP biogenesis is essential for cell growth and proliferation in eukaryotes because snoRNPs are involved in pre-rRNA modification, which in turn regulates ribosome biogenesis (21–23). There are two major classes of snoRNAs – box C/D and box H/ACA snoRNAs – which function as guide RNA, targeting snoRNPs to specific nucleotides on pre-rRNA for enzymatic modifications, such as 2′-*O*-methylation and pseudouridylation, respectively (Figure 2) (24, 25). Recent studies have shown that the R2TP functions as a chaperone for the both box C/D and box H/ACA snoRNP assembly (16, 17), and it regulates ribosome biogenesis and subsequently controls cell proliferation (16, 26). Intriguingly, it has been reported that the expression levels of some components of R2TP, box C/D snoRNP, as well as box H/ACA snoRNP, are deregulated in various cancer cells (described in main section). On the

***Corresponding author: Yoshito Kakihara,** Division of Dental Pharmacology, Department of Dentistry, Niigata University, 2-5274 Gakkocho-dori, Chuo-ku, Niigata, 951-8514, Japan, e-mail: kakihara@dent.niigata-u.ac.jp
**Makio Saeki:** Division of Dental Pharmacology, Department of Dentistry, Niigata University, 2-5274 Gakkocho-dori, Chuo-ku, Niigata, 951-8514, Japan

**Table 1** The components of R2TP in yeast and human.

| Yeast | Human | Features |
|---|---|---|
| Rvb1 | RuvBL1/Pontin/TIP49 | AAA+ superfamily |
| Rvb2 | RuvBL2/Reptin/TIP48 | AAA+ superfamily |
| Pih1/Nop17 | PIH1D1 | PIH1 domain-containing |
| Tah1 | RPAP3 | TPR domain-containing |

basis of recent data, we discuss how the R2TP contributes to carcinogenesis through regulating snoRNP biogenesis.

## R2TP function in box C/D snoRNP biogenesis

The box C/D snoRNP is composed of box C/D snoRNA and the core proteins, Nop1 (fibrillarin in human), Snu13 (15.5K in human), Nop56, and Nop58 (21) (Figure 2). This RNA-protein complex is involved in 2′-*O*-methylation of the pre-rRNA. The snoRNA moiety functions as guide RNA that forms base pairs with pre-rRNA, which in turn allows the pre-rRNA to be methylated by Nop1/fibrillarin. This modification is essential for the subsequent endonucleotic cleavage and processing of the pre-rRNA to produce mature rRNAs (21, 27). The assembly of snoRNP is hierarchical and its proper assembly is essential for both the activity and stability of snoRNP (6, 16, 17, 28–31). It has been proposed that the assembly of box C/D snoRNP starts with the interaction between box C/D snoRNA and Snu13/15.5k, followed by the binding of Nop58 and then Nop1/fibrillarin and Nop56 are recruited (28, 32). In this process, Nufip/Rsa1 (snoRNP assembly factor) and Rvbs facilitate the assembly of box C/D snoRNP by bridging the interactions of core components of box C/D snoRNP: between 15.5K and both Nop56 and Nop58 (29–31). Nufip is likely to regulate the interaction between Rvbs and the box C/D core proteins by tightly interacting with 15.5K (30). An involvement of R2TP in this assembly process also have been identified in yeast and human (6, 29), and very recently, it was shown that the yeast R2TP specifically associates with unassembled Nop58 which has yet to be assembled with other core factors such as Nop1, Snu13, and Nop56 (16). Under nutrient-rich condition, the yeast R2TP localizes to nucleus and tightly associates with the unassembled Nop58 that stabilizes Nop58. However, under the nutrient-limiting condition, it dissociates from Nop58 and subsequently delocalizes to cytoplasm. Importantly, Pih1 is a key factor in this process. When the yeast

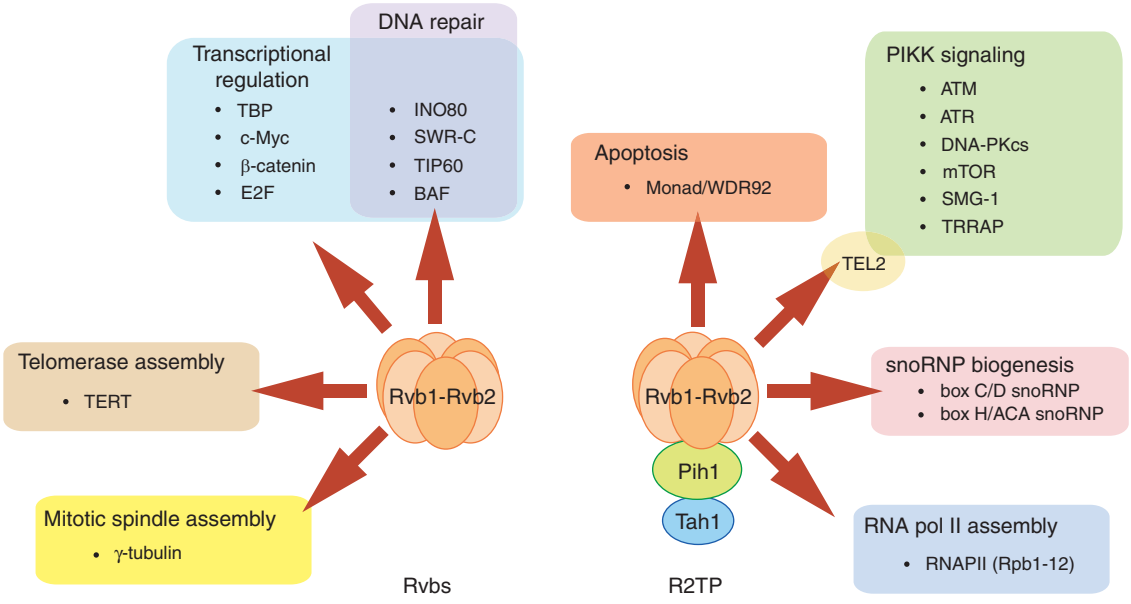


**Figure 1** Functions of Rvbs (Rvb1-Rvb2) and R2TP complexes.
Rvbs are involved in transcriptional regulation, DNA repair, telomerase assembly, and mitotic spindle assembly [reviewed in (3, 5, 12)]. In contrast, R2TP is involved in apoptosis, PIKK signaling (functions in stress responses including DNA repair, transcription, and mRNA decay), and snoRNP biogenesis [reviewed in (2, 4)]. This change in functional profile of the Rvbs is achieved by Pih1/PIH1D1 assisting the binding of Rvbs and Tah1/RPAP3 to the specific targets such as Nop58 (box C/D snoRNP), dyskerin (box H/ACA snoRNP), Rpb1 (RNA polymerase II), and Tel2 [TTT (Tel2-Tti1-Tti2) complex, which mediates interaction with PIKK complexes]. Target proteins/complexes of Rvbs or R2TP in each cellular process are shown in the box.

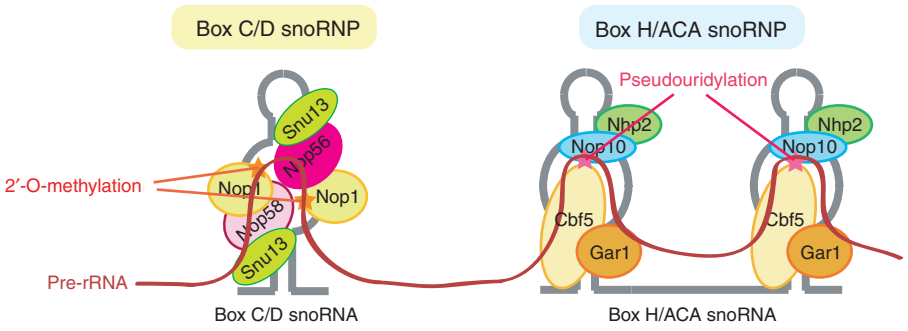


**Figure 2** Components of the box C/D and box H/ACA snoRNP.
Mature form of box C/D and box H/ACA snoRNPs are shown (21). The box C/D snoRNP is composed of box C/D snoRNA and four core proteins: Snu13, Nop1/fibrillarin, Nop56, and Nop58, and is required for 2′-*O*-methylation of pre-rRNA (21, 23). Nop1 is the catalytic subunit of this complex. The box H/ACA snoRNP is composed of box H/ACA snoRNA and four core proteins – Nhp2, Gar1, Nop10, and Cbf5/dyskerin – and is involved in pseudouridylation of pre-rRNA (21, 23). The box H/ACA snoRNA includes two hairpins and each of these associates with the core protein factors. Cbf5/dyskerin is the catalytic subunit of this snoRNP.

Pih1 was artificially tagged with a nuclear export signal and thus was forced to localize only in the cytoplasm, Nop58 protein level was significantly reduced, and cell growth was also severely compromised (16).

Altogether, the current evidence shows that the yeast and human R2TP facilitates/regulates the assembly of box C/D snoRNPs at its very early stage. Intriguingly, the activity and subcellular localization of the yeast R2TP are tightly regulated by growth conditions. During the process, the association of yeast Pih1 with free form of Nop58 is essential and appears to have a direct effect on cell growth (16). However, details on how the R2TP-Nop58 interaction contributes to the following maturation process of box C/D snoRNP remain to be elucidated. Also, it has yet to be determined whether subcellular localization of R2TP and interaction between R2TP and Nop58 in mammalian cells are altered under the different growth conditions.

## R2TP function in box H/ACA snoRNP biogenesis

The box H/ACA snoRNP consists of box H/ACA snoRNA, and the core proteins, Nhp2, Gar1, Nop10, and Cbf5 (referred to as dyskerin, NAP57, and DKC1 in human) (21) (Figure 2). Like box C/D snoRNP, box H/ACA snoRNP is also involved in modification of pre-rRNA, which is required for downstream processing. The Cbf5/dyskerin functions as a pseudouridine synthase, thus this RNA-protein complex is responsible for pseudouridylation of the target uridines on the pre-rRNA (33, 34). It has been shown that the box H/ACA snoRNP assembly occurs co-transcriptionally and requires assembly factors, which are not integral components of the mature box H/ACA snoRNP, such as Naf1 and Shq1 (35–37). The Shq1 initially interacts with free form of Cbf5/dyskerin, which has yet to be co-transcriptionally assembled with box H/ACA snoRNA and other core proteins (36, 38). In mammalian systems, R2TP subsequently interacts directly with Shq1-dyskerin complex, resulting in the latter's dissociation (17). Then, another assembly factor Naf1 interacts with dyskerin and the complex is brought to the transcription sites of box H/ACA snoRNA. Next, Naf1 is replaced by Gar1 to form the mature snoRNP complex by assembling with other factors such as Nhp2, and Nop10 (39). Additionally, given that all core protein factors of box H/ACA snoRNP also assembles with TERC (telomerase RNA component), forming TERC-containing RNP (34), its biogenesis could follow that of canonical box H/ACA snoRNPs by involving R2TP (17). In yeast, Rvbs are involved in box H/ACA snoRNP biogenesis (6, 40), although the contribution of Pih1 and Tah1 to the process, if any, remains unknown.

## Deregulation of snoRNA biogenesis in tumorigenesis

A common feature of the cancer cells is abnormally enlarged nucleolus, as a result of hyperactive ribosome biogenesis (41). Consequently, cellular processes that are related to ribosome biogenesis, such as the biosynthesis of ribosomal proteins, rRNA, snoRNAs and core proteins of snoRNPs, are expected to be highly activated (41–44). In support of this argument, the deregulation of snoRNA/snoRNP biogenesis has been reported in various cancer cell types [reviewed in (45)]. Given that rRNA biosynthesis

is upregulated in cancer cells, it is reasonable to assume that the expression of snoRNAs will also be upregulated, as they are required for pre-rRNA processing. Nevertheless, while some snoRNAs are indeed upregulated in cancer cells, others are actually downregulated. For example, Mannoor et al. investigated snoRNAs expression in tumor-initiating cells (TICs; also known as cancer stem cells) of non-small cell lung cancer (46). They identified 22 snoRNAs that showed changes in expression by more than threefold in TICs compared to non-TICs. Of these 22 snoRNAs, 21 were upregulated, but one was downregulated (46). Among the upregulated snoRNAs, SNORA42 showed the highest expression. Intriguingly, in TIC xenografts of mice, knock down of SNORA42 resulted in a decrease of tumorigenesis (46). In other cancer cell lines, it has been identified that SNORD33, SNORD44, SNORD66, and SNORD76 are upregulated, and a depletion of their expression decreases both cancer cell growth and colony formation (47, 48). Regarding snoRNA downregulation that is related to tumorigenesis, human U50 box C/D snoRNA (also called SNORD50) is mutated and downregulated in prostate and breast cancers (49, 50). On the other hand, U50 overexpression inhibits colony formation of prostate and breast cancers, suggesting that the U50 functions as a tumor suppressor (49, 50). In mice, deletion of U50 snoRNA decreased methylation levels of the target rRNA, however, no significant growth phenotype or tumorigenesis were observed. Subtle effects on lymphoid organs such as splenomegaly and swollen lymph nodes were observed more frequently compared to wild-type mice (51). Additionally, other snoRNAs (SNORD43, SNORD44 and SNORD48) are known to have decreased expression levels in cancer cells, although it mechanism has not been elucidated (52).

## Potential role of R2TP in tumorigenesis through snoRNP pathway

As mentioned above, R2TP is involved in various cellular processes and exerts chaperone function, assisting the assembly of multiprotein complexes via interaction with their specific subunits. As reviewed by Grigoletto et al. (53), RuvBL1/pontin and RuvBL2/reptin are known to be involved in carcinogenic processes of hepatocellular carcinoma as well as colorectal cancer. Although the accumulating data indicates the involvement of RuvBL1/pontin and RuvBL2/reptin in carcinogenesis, the underlying mechanism remains largely unknown. Several reports suggest that the regulatory role of Rvbs/R2TP in snoRNP biogenesis is associated with tumorigenesis. It has been shown that Rvbs and Pih1 as well as Nop56 are co-immunoprecipitated with Nop58 in Hela cells (31). In addition, PIH1D1 depletion resulted in significant decrease of box C/D snoRNAs and accumulation of U3 box C/D snoRNA in Cajal body where snoRNPs transit during its maturation process (31). Furthermore, Su et al. showed that snoRNA/snoRNP biogenesis involving RuvBL1/pontin is enhanced in breast cancer (54). They observed that the depletion of RuvBL1/pontin markedly reduced box C/D snoRNA accumulation, which is significantly upregulated in breast cancer cells. Also, similar effects were observed by impairment of box C/D snoRNP core factors such as fibrillarin, NOP56, and NOP58, suggesting that RuvBL1/pontin and box C/D snoRNP cooperatively contribute to tumorigenesis. Moreover, they showed that a suppression of snoRNA biogenesis compromised tumorigenicity through activating p53 (54).

In addition to snoRNAs, the upregulation of R2TP has also been reported in various cancer cells as shown in Table 2. For example, in human hepatocellular carcinoma (HCC), the mRNA and/or protein levels of RuvBL1/pontin and RuvBL2/reptin are increased as compared to non-tumor liver (55–60). Also, it has been shown that RuvBL1/pontin is overexpressed in breast, colon, and colorectal cancers (61–64) and RuvBL2/reptin is overexpressed in breast, colon, gastric, and renal cell cancers (62, 65–67). Additionally, it has been reported that RPAP3 and PIH1D1 expressions are upregulated in breast cancer as well as colon cancer (62, 68). As aforementioned, R2TP directly targets both Nop58 and dyskerin, which are a component of box C/D and box H/ACA snoRNP, respectively. Interestingly, it has been observed that the expression of the both Nop58 and dyskerin are increased

**Table 2** The upregulation of R2TP in various cancers.

| Upregulated genes | Types of cancer | References |
|---|---|---|
| RuvBL1/Pontin | Hepatocellular carcinoma | (55, 57, 60) |
| | Breast cancer | (62) |
| | Colon cancer | (61, 62) |
| | Colorectal cancer | (63, 64) |
| RuvBL2/Reptin | Hepatocellular carcinoma | (55, 56, 58–60) |
| | Breast cancer | (62, 66) |
| | Colon cancer | (62) |
| | Gastric cancer | (65) |
| | Renal cell cancer | (67) |
| RPAP3 | Breast cancer | (62) |
| | Colon cancer | (62) |
| PIH1D1 | Breast cancer | (62, 68) |
| | Colon cancer | (62) |

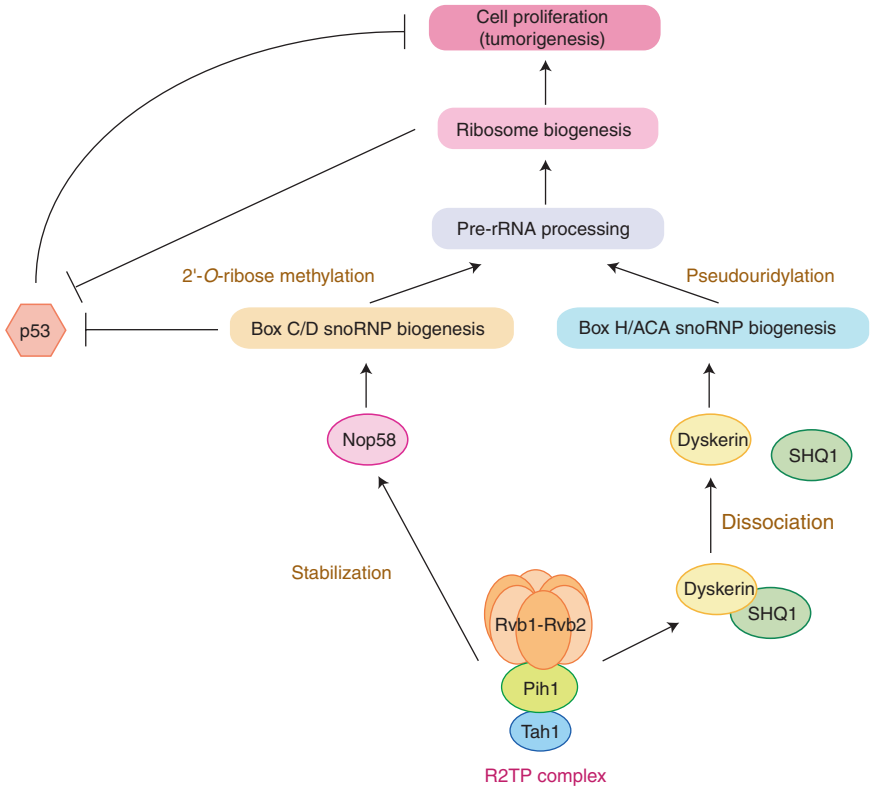


**Figure 3** Involvement of R2TP in cell proliferation/tumorigenesis through snoRNP biogenesis.
R2TP regulates both box C/D and box H/ACA snoRNP biogenesis, and subsequently controls pre-rRNA processing, ribosome biogenesis, and cell proliferation. Elevated snoRNP biogenesis inactivates p53 activity and triggers tumorigenesis, whereas inhibition of snoRNP biogenesis activates p53 and inhibits the cell proliferation.

in some cancer cells. For Nop58, the expression level was compared between non-metastic and metastatic melanoma cells from mice, and its expression was significantly increased in metastatic cells (69). Also, in tumor samples of human malignant melanoma, Nop58 is upregulated specifically in metastatic group compared to the non-metastatic group (69). For dyskerin, although downregulation of the expression has been observed in a subset of tumors (70), upregulated expression was determined in a number of human cancer cells and also its overexpression is correlated with aggressive proliferation of the tumor cells such as hepatocellular (71), colon (72), prostate (73, 74), head and neck carcinomas (75). Given that the both Nop58 and dyskerin are unstable proteins and associated/stabilized by R2TP (16, 17, 76), the expression level of R2TP components also might be upregulated to maintain the target proteins level in above-mentioned cancers.

In summary, it is shown how R2TP function is related to tumorigenesis through snoRNP biogenesis (Figure 3). R2TP directly interacts with Nop58 and dyskerin, a component of box C/D and box H/ACA snoRNP (16, 17). R2TP stabilizes Nop58 protein level, and facilitates the assembly with other core components (16). For dyskerin, R2TP dissociates it from the associated protein Shq1 to promote the following assembly process (17). In cancer cells, some of the R2TP and snoRNP components as well as many snoRNAs are upregulated to enhance the snoRNP synthesis to process the pre-rRNA efficiently and to produce high amount of ribosome. This elevated snoRNP and ribosome biogenesis negatively regulates the tumor suppressor p53 and that triggers tumorigenesis, whereas inhibition of the snoRNP synthesis in cancer cells induces ribosome stress, which activates p53 and inhibits cell proliferation (54). In this context, R2TP is a crucial regulator of cell proliferation. To downregulate the highly activated snoRNP/ribosome biogenesis in cancer cells, inhibition of the ATPase activity of R2TP and/or blocking its interaction with Nop58, and dyskerin could be considered a promising therapeutic approach. Thus, R2TP has great potential as a drug target for future cancer therapeutic research and development.

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## List of abbreviations

| | |
|---|---|
| snoRNA | small nucleolar RNA |
| snoRNP | small nucleolar ribonucleoprotein |
| PIKK | phosphatidylinositol 3-kinase-related kinase |
| rRNA | ribosomal RNA |

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