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CELL BIOLOGY

The Emergence of the Conserved AAA+ ATPases Pontin and Reptin on the Signaling Landscape

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Pontin (also known as RUVBL1 and RVB1) and Reptin (also called RUVBL2 and RVB2) are related members of the large AAA+ (adenosine triphosphatase associated with diverse cellular activities) superfamily of conserved proteins. Various cellular functions depend on Pontin and Reptin, mostly because of their functions in the assembly of protein complexes that play a role in the regulation of cellular energetic metabolism, transcription, chromatin remodeling, and the DNA damage response. Little is known, though, about the interconnections between these multiple functions, how the relevant signaling pathways are regulated, whether the interconnections are affected in human disease, and whether components of these pathways are suitable targets for therapeutic intervention. The First International Workshop on Pontin (RUVBL1) and Reptin (RUVBL2), held between 16 and 19 October 2012, discussed the nature of the oligomeric organization of these proteins, their structures, their roles as partners in various protein complexes, and their involvement in cellular regulation, signaling, and pathophysiology, as well as their potential for therapeutic targeting. A major outcome of the meeting was a general consensus that most functions of Pontin and Reptin are related to their roles as chaperones or adaptor proteins that are important for the assembly and function of large signaling protein complexes.

Introduction

Pontin (also known as RUVBL1, RVB1, Tip49a, ECP-54, Tih1, p50, and Tap54β) and Reptin (RUVBL2, RVB2, Tip49b, ECP-51, Tih2, p47, and Tap54α) came to attention in the late 1990s with their cloning and identification as putative DNA helicases with homology to bacterial RuvB (1, 2). They belong to the AAA+ [adenosine triphosphatase (ATPase) associated with various cellular activities] superfamily of proteins, members of which are characterized by the presence of conserved Walker A and B sequences that are involved in ATP binding and hydrolysis, respectively. It subsequently became clear that these proteins play diverse roles in many essential cellular processes. Because Pontin and Reptin help assemble complexes containing members of the phosphati-

dylinositol-3 kinase-related kinase (PIKK) family, these proteins are involved in many signaling pathways, including those regulating nutrient sensing, RNA metabolism, and DNA damage repair. How can Pontin and Reptin be involved in the assembly of protein complexes in the cytoplasm as well as the remodeling of chromatin in the nucleus? Although they are most often present in the same complexes, Pontin and Reptin can have opposing functions, notably in the regulation of transcription. Controversies have arisen regarding the nature of their oligomeric organization, which is likely the key to understanding the functions of these proteins. Some of these issues have been discussed in several recent reviews (3–6). The First International Workshop on Pontin (RUVBL1) and Reptin (RUVBL2), held near Bordeaux,

Meeting Information: The First International Workshop on Pontin (RUVBL1) and Reptin (RUVBL2) took place at the European Institute for Chemistry and Biology in Pessac, France, 16 to 19 October 2012.

France, brought together ~60 scientists from 14 countries. At the meeting, these researchers discussed many aspects of Pontin and Reptin structure and function. The meeting was organized by Jean Rosenbaum (Université de Bordeaux, Bordeaux, France), Otmar Huber (Jena University Hospital, Jena, Germany), and Ted Hupp (University of Edinburgh, Edinburgh, UK).

One important objective of the meeting was to adopt a common terminology for referring to Pontin and Reptin, because each is known by up to 10 different names. Though this proved to be a difficult task, a consensus was reached to restrict each to three names: the HUGO terms RUVBL1 and RUVBL2; Pontin and Reptin, originally coined by researchers in the *Drosophila melanogaster* community and favored by many; and RVB1 and RVB2, the names preferred by those using yeast as a model system. For clarity, only Pontin and Reptin will be used in this Meeting Report.

Anindya Dutta (University of Virginia, Charlottesville, Virginia, USA) offered an overview of the field, pointing out that many groups have described Pontin and Reptin based on their association with other proteins of interest. Pontin and Reptin have now been implicated in functions related to chromatin remodeling complexes, like INO80 or NuA4; stimulation or repression of transcription factors, such as Myc or β-catenin; and assembly of ribonucleoprotein complexes, like small nucleolar ribonucleoproteins (snoRNPs) and telomerase. Consistent with their role as assembly factors or chaperones, Pontin and Reptin are associated with heat shock protein 90 as part of the R2TP complex. Pontin and Reptin have also been implicated in cell transformation, the DNA damage response, apoptosis, and mitosis. After this introduction, Dutta summarized work from his own laboratory demonstrating that the ATPase activities of Pontin and Reptin were equally and independently essential for viability in the budding yeast *Saccharomyces cerevisiae*. Pontin and Reptin were an integral part of the yeast INO80 complex and are required for its chromatin remodeling activity, most likely because they are required to

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incorporate actin-related protein 5 (ARP5) into the complex (7). Dutta reported that human Pontin and Reptin were also associated with one another and protected each other from degradation. Further, Pontin and Reptin are part of the multisubunit NuA4 remodeling complex, which plays a key role in both transcription and DNA repair.

Key subunits of NuA4 include Reptin and Pontin, the acetyltransferase TIP60, and the motor ATPase p400. The Pontin and Reptin subunits of NuA4 are essential for the acetyltransferase activity of the TIP60 subunit during DNA repair (8) and also contribute to the assembly and structural organization of the NuA4 complex (9). Knocking down

Pontin and Reptin in cultured human cells decreased the acetyltransferase activity of the NuA4 complex, leading to increased longevity and abundance of the phosphorylated form of the histone H2AX after DNA damage. Dutta concluded by enumerating important unanswered questions: Do Pontin and Reptin always work together, or can they function independently in different complexes? Are their ATPase activities required for all their functions? Do they work as hexamers, dodecamers, or simply as dimers?

Biochemistry and Structural Biology

After several years of conflicting results, structural data on human Pontin and Reptin complexes are now converging. Pedro Matias (Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Oeiras, Portugal) presented x-ray crystallographic three-dimensional structures of Pontin in complex with Reptin (Fig. 1). Pontin forms hexamers, and each monomer is composed of three domains, with domains I and III cooperating in ATP binding and domain I mediating ATP hydrolysis. The role of domain II is still poorly understood but likely involves interactions with other proteins, DNA, or RNA. The ATPase and helicase activities of Pontin are weak, probably due to a tight-binding ATP pocket, which requires conformational changes to become active (10). Crystals of the Pontin-Reptin complex, obtained only after truncation of the central (second) domain of each protein, revealed a dodecameric structure formed by two heterohexameric rings that contact one another through the truncated second domains. Surprisingly, Pontin and Reptin variants with a truncated domain II displayed greater ATPase and helicase activities than the full-length proteins, supporting the hypothesis that these activities are regulated by domain II, presumably through interactions with other binding partners (11).

In parallel, Oscar Llorca's lab (Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, Madrid, Spain) used cryogenic electron microscopy to obtain a structure of dodecameric complexes of full-length human Pontin and Reptin that proved very similar to that from Matias's lab (12). In addition, Llorca's team demonstrated the coexistence of different conformations of the dodecamer within the same preparation, which might affect the ATPase activity. Key residues likely to be required for conformational changes were

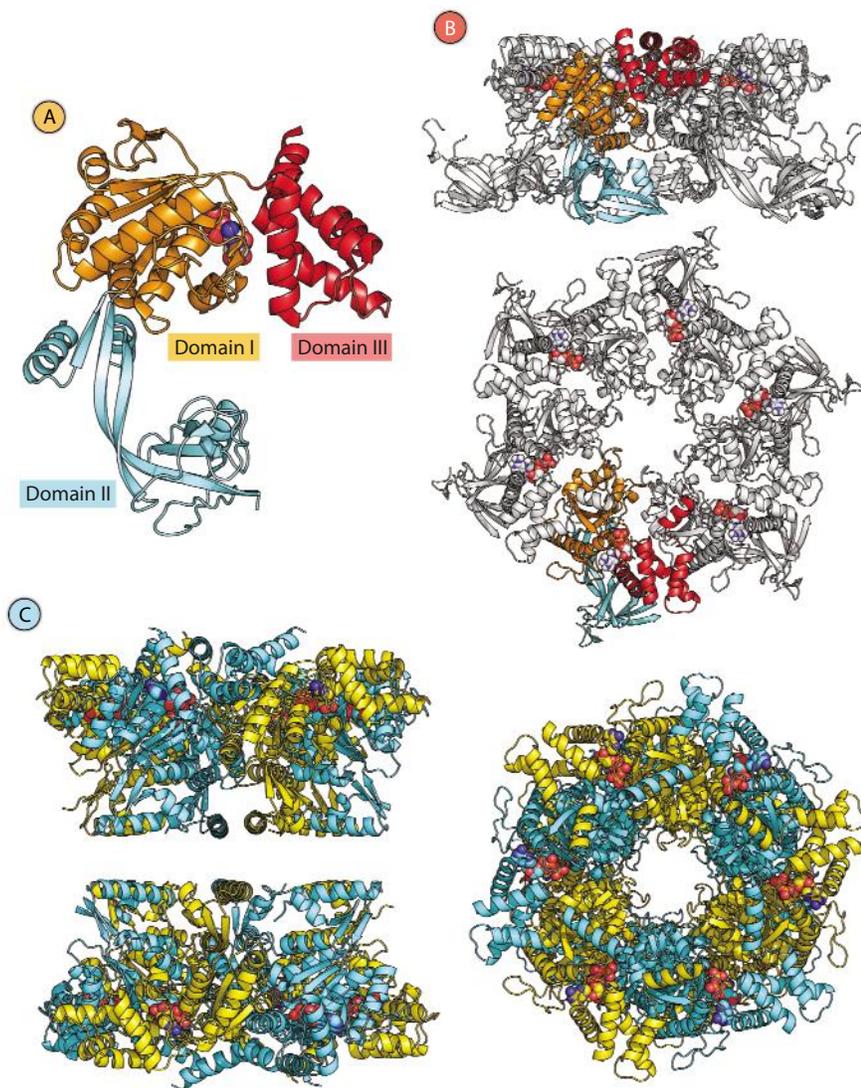


Fig. 1. Structures of the Pontin monomer, the Pontin hexamer, and the Pontin-Reptin dodecamer. **(A)** Ribbon diagram of the Pontin monomer [Protein Data Bank identification number (PDB ID) 2c9o] showing its domain structure. Domains I and III are involved in binding and hydrolysis of ATP, whereas the function of domain II likely involves interactions with protein partners, DNA, or RNA. A molecule of adenosine diphosphate (ADP) is shown bound to Domain I. **(B)** Ribbon diagrams of the Pontin hexamer (PDB ID 2c9o). The "side" (upper diagram) and "top" (lower diagram) views are related by a 90° rotation about the horizontal axis. One monomer is shaded according to the domain structure as represented in (A). **(C)** Ribbon diagrams of the Pontin-Reptin dodecamer with truncated domain II (PDB ID 2xsx). Pontin monomers are shown in yellow; Reptin monomers are shown in light blue. The two views are 90° apart about a horizontal rotation axis. The ADP ligands in panels A and B and the ATP ligands in panel C are represented as spheres with gray for carbon, blue for nitrogen, red for oxygen, and orange for phosphorus.

identified by Mikhail Grigoriev (Université Paul Sabatier, Toulouse, France), Mikhail Petukhov (Saint Petersburg State Polytechnical University, Saint Petersburg, Russia), and their collaborators with the use of molecular dynamics simulations (13). Further analysis of alternative conformations of Pontin and Reptin should contribute to a better understanding of their extraordinarily diverse biological functions.

Nuclear Functions: Transcription and Chromatin Remodeling

Kai Albring and Otmar Huber (Jena University Hospital, Jena, Germany) demonstrated multiple roles of Pontin and Reptin in transcriptional regulation. Albring and Huber previously reported that within the nucleolus, Pontin associates with RNA polymerase I and forms a complex at regulatory sites of ribosomal RNA transcription with c-Myc (14). They now report that the estrogen receptor forms complexes with Pontin or Reptin, or both, at regulatory cis elements in a gene-specific manner. The interaction of Reptin with the estrogen receptor provided Mathieu Dalvai (Laboratoire de Biologie Moléculaire Eucaryote, Université Paul Sabatier, Toulouse, France) with a means to investigate how Reptin is involved in the positive regulation of gene expression through the control of chromatin structure. He showed that the histone variant H2A.Z is present at the transcription start site and at downstream enhancer sequences of the *cyclin D1* gene (*CCND1*) when the gene is poorly transcribed (15). Reptin promoted the release of H2A.Z, which, in turn, reduced the frequency of enhancer-promoter contacts, thereby releasing a repressive intragenic loop and enabling the estrogen receptor to bind the *CCND1* promoter.

Sung Hee Baek's group (Seoul National University, Seoul, South Korea) studied the role of Pontin and Reptin in the regulation of transcription in the context of cancer. Baek's lab showed how posttranslational modifications of Pontin and Reptin affect their transcriptional activities and proposed a model in which these proteins can have roles independent of one another in the regulation of gene expression. For example, Baek's team showed that a specific TIP60-Pontin complex (which is distinct from the previously described NuA4 complex) promotes expression of the metastasis suppressor *KAI1* in nonmetastatic prostate cancer cells, whereas a β -catenin-Reptin complex represses expression of *KAI1* in metastatic

cells. Sumoylation of Reptin further stimulates the repressive potential of Reptin by promoting its nuclear localization and increasing its interaction with the histone deacetylase HDAC1 (16, 17), whereas sumoylated Pontin transcriptionally activates androgen receptor-responsive genes and, consequently, increases the proliferation of prostate cancer cells (18). The group also reported that Reptin and Pontin are modified by the lysine methyltransferase G9a during hypoxia. Lysine-methylated Reptin participates in the repression of genes involved in hypoxic signaling and inhibits tumor growth in vivo (19), whereas lysine-methylated Pontin increases the transcriptional activity of the α subunit of hypoxia-inducible factor 1 (HIF1 α) and enhances cell proliferation. Genome-wide analysis revealed that Pontin- and Reptin-dependent target genes do not overlap in the cellular response to hypoxia (20).

Esther Marza (University of Bordeaux, Bordeaux, France) studied the regulation of transcription in the context of endoplasmic reticulum (ER) stress. In an in vivo genome-wide RNA interference screen, Marza's group showed that *Caenorhabditis elegans* Reptin functionally interacts with the AAA+ family member cell-division cycle protein 48 (CDC48) during ER stress. Marza's team suggests that CDC48-dependent degradation of Reptin is required to release the inhibition of transcription of ER stress target genes. This mechanism was conserved in mammalian cells.

As mentioned previously, Reptin and Pontin are components of the mammalian NuA4 complex, which plays a key role in regulating DNA repair. Brendan Price (Dana-Farber Cancer Institute, Boston, Massachusetts, USA) demonstrated that the NuA4 remodeling complex exchanged H2A.Z for H2A onto nucleosomes at sites of DNA damage before double-strand break repair. Exchange of H2A.Z required two subunits of NuA4, TIP60 and p400, and was essential for correct processing and repair of DNA damage (21). Furthermore, both the Pontin and Reptin subunits of NuA4 were critical for H2A.Z exchange and played an unexpected role in regulating the activity of the p400 and TIP60 subunits of NuA4, similar to the observed regulation of NuA4 by Reptin and Pontin reported by the Dutta group.

In addition to functioning as a component of NuA4, several isoforms of TIP60 exist that function independently of NuA4 but

whose function remains poorly understood. John Lough (Medical College of Wisconsin, Milwaukee, Wisconsin, USA) showed that adult cardiomyocytes exclusively express the TIP60 β isoform (22). He then showed that upon stress imposed by either overexpression of c-Myc or aortic banding, cell cycle markers increased in cardiomyocytes of TIP60^{+/+} adult mice, consistent with the suggested role for TIP60 in cell cycle regulation (23).

Finally, Wolfram Antonin (Friedrich Miescher Laboratory of the Max Planck Society, Tübingen, Germany) identified yet another function for Pontin and Reptin. He showed that both are required for chromatin decondensation at the end of mitosis in both *Xenopus laevis* egg extracts and in human HeLa cells. These data indicate that chromatin decondensation is an ATP-dependent process requiring specific cellular machinery rather than the mere inactivation of condensation factors.

Protein-Protein Complex Assembly and Molecular Chaperoning

Walid A. Houry (University of Toronto, Toronto, Canada) presented an overview of the R2TP complex that his group identified (24). This complex consists of Pontin, Reptin, and two heat shock protein 90 (Hsp90) partners: tetratricopeptide repeat domain-containing protein associated with Hsp90 [Tah1 in yeast; known as RNA polymerase II associated protein 3 (RPAP3) or Spagh in mammalian cells] and protein interacting with Hsp90 [Pih1 in yeast; also called nucleolar protein 17, but PIH1 domain-containing 1 (PIH1D1) in mammalian cells]. Makio Saeki (Osaka University, Osaka, Japan) identified the presence of three isoforms of RPAP3 in mammalian cells. Saeki showed that only isoform 1 is part of the R2TP complex and stabilizes PIH1D1 (25). Houry also presented the recently solved nuclear magnetic resonance structure of Tah1 (26). His group, in collaboration with Joaquin Ortega's group (McMaster University, Hamilton, Canada), showed that yeast Pontin and Reptin form a heterohexameric complex (27, 28). A pathway for the assembly of the box C/D class of snoRNPs, as mediated by Hsp90-R2TP, was proposed. In parallel, Tom Meier (Albert Einstein College of Medicine, New York, USA) provided important clues about the molecular role of Pontin and Reptin in the biogenesis of the H/ACA class of RNPs that includes telomerase, showing that Pontin

and Reptin function in separating the pseudouridine synthase NAP57 (dyskerin) from the assembly factor SHQ1 (29).

Bérenère Pradet-Balade and colleagues (Institut de Génétique Moléculaire, Montpellier, France) have also demonstrated a role for the R2TP complex in snoRNP assembly (30) and on the cytoplasmic assembly of RNA polymerase II (31). They now show that the pathway is conserved in *Drosophila*, establishing it as a powerful system to investigate new R2TP clients.

Shigeo Ohno (Yokohama City University School of Medicine, Yokohama, Japan) showed that Pontin and Reptin, together with the other R2TP components, interact with many PIKK family members, including ataxia telangiectasia mutated (ATM), ataxia telangiectasia Rad3-related (ATR), DNA-dependent protein kinase catalytic subunit (DNA-PKcs), suppressor with morphogenetic effect on genitalia-1 (SMG-1), transformation/transcription domain-associated protein, and the mechanistic target of rapamycin (mTOR) (32). Depletion of Pontin or Reptin in cultured human cells destabilizes PIKKs, leading to dramatic phenotypic effects—notably, a defect in SMG-1-dependent nonsense-mediated mRNA decay. Data published after this meeting show that Pontin and Reptin are also required for the association of mTOR with lysosomes (33). The central position of PIKKs in several major signaling pathways establishes Pontin and Reptin as important actors in cell signaling (Fig. 2).

Although separate from their chromatin modification and transcriptional regulatory functions, it is clear that the chaperone functions of Pontin and Reptin also probably affect nuclear processes due to their role in the assembly of nuclear complexes, such as INO80 and NuA4-TIP60, as highlighted by Anindya Dutta. Whether this only happens when Pontin and Reptin are part of the R2TP complex and whether R2TP also has clients in addition to those already identified remain open questions.

Pathophysiology and Therapeutic Targeting

Jean Rosenbaum's team (University of Bordeaux, Bordeaux, France) demonstrated that the genes encoding Pontin and Reptin were overexpressed in human liver cancer, where they were required for growth and viability of tumor cells both in vitro and in vivo (34–36). Rosenbaum's group now shows that the ATPase activity of Reptin is required for sustaining tumor cell growth, suggesting that

Binding partners	mTOR	SMG-1	ATM	ATR	DNA-PKcs
Complex	mTORC1	SURF	ATR/ATRIP		
Signaling	p70S6K	Upf1	Chk2	Chk1	
Outcome	Translation, energy metabolism	Nonsense-mediated mRNA decay	DNA damage response, transcription		

Fig. 2. Pontin and Reptin as major actors in cell signaling. Pontin and Reptin are required for the stabilization of every PIKK family member and for the assembly of supramolecular complexes involving PIKKs. Their functional role has been established by showing decreased activation of PIKK effectors upon Pontin or Reptin silencing, with consequences on biological outcomes. Pontin and Reptin help assemble the mTOR complex 1 (mTORC1), thus modulating signaling through the p70 ribosomal S6 kinase (p70S6K) and thereby affecting cellular processes that include protein translation and energy metabolism. Similarly, Pontin and Reptin stabilize SMG-1 that will be incorporated into the SMG-1, Upf1, eRF1, and eRF3 (SURF) complex, enabling SMG-1 to phosphorylate Upf1 and leading to nonsense-mediated decay of mRNAs. Pontin and Reptin also stabilize ATM, ATR, and DNA-PKcs and help assemble the ATR-ATR interacting protein (ATR-ATRIP) complex, which promotes DNA damage response signaling.

Reptin could be a viable drug target for cancer therapy (37). In collaboration with Michel Laguerre's group (Institut Européen de Chimie et de Biologie, Pessac, France), Patrick Lestienne, from the Rosenbaum group, presented small molecule inhibitors of Pontin's ATPase activity identified through in silico modeling and in vitro testing (38).

Pontin and Reptin are required for the assembly of telomerase (39, 40). An alternative mechanism (ALT) to maintain telomeres instead of telomerase activity, already long known to be used in some tumor cells, has recently been shown to occur in normal mammalian somatic cells also (41). Klaus Holzmann (Medizinische Universität Wien, Vienna, Austria) reported that *Pontin*, but not *Reptin*, transcripts were reduced in ALT as compared with non-ALT tumor cell lines. Although *Pontin* was generally overexpressed in colorectal cancer (CRC) (42), preliminary data from human specimens of CRC with ALT showed reduced expression of *Pontin* in tumors compared with nontumor tissues. This change may influence the activity of Pontin- and Reptin-containing complexes in several contexts—notably in the DNA damage response.

Oxana Bereshchenko (University of Perugia, Italy) reported on the function of Pontin in an intact mammalian organism. Deletion of *Pontin* in mice resulted in embryonic lethality due to a loss of embryonic stem (ES) cell function. The defect was not characterized further, but no *Pontin* knock-

out colonies were detected in ES cell outgrowth cultures in vitro. Conditional ablation of *Pontin* in hematopoietic tissues led to bone marrow failure characterized by the apoptotic loss of hematopoietic stem cells in adult mice. Thus, Pontin is critical for the function of both embryonic stem cells and adult hematopoietic stem cells (43). Further in vivo studies will be important to better define the physiological and pathophysiological functions of Pontin and Reptin.

Ted Hupp (University of Edinburgh, Edinburgh, UK) previously demonstrated an interaction between anterior gradient-2 (AGR2) and Reptin (44). AGR2 is a component of a pro-oncogenic signaling pathway that mediates cellular transformation, ER homeostasis, and p53 inhibition. Hupp demonstrated an interaction between AGR2, Reptin, and p53 that is mediated by the p53 tetramerization domain and stimulated by AGR2. This interaction defines a previously unidentified pathway for suppressing the activity of p53.

In contrast to Pontin and Reptin, the two RuvB-like proteins found in humans and yeast, the malaria parasite *Plasmodium falciparum* contains three RuvB-related proteins. A phylogenetic analysis presented by Moaz Ahmad (International Centre for Genetic Engineering and Biotechnology, New Delhi, India) revealed that PfRuvB1 and PfRuvB2 are most similar to Pontin, whereas PfRuvB3 is more closely related to Reptin (45). Further studies are required to

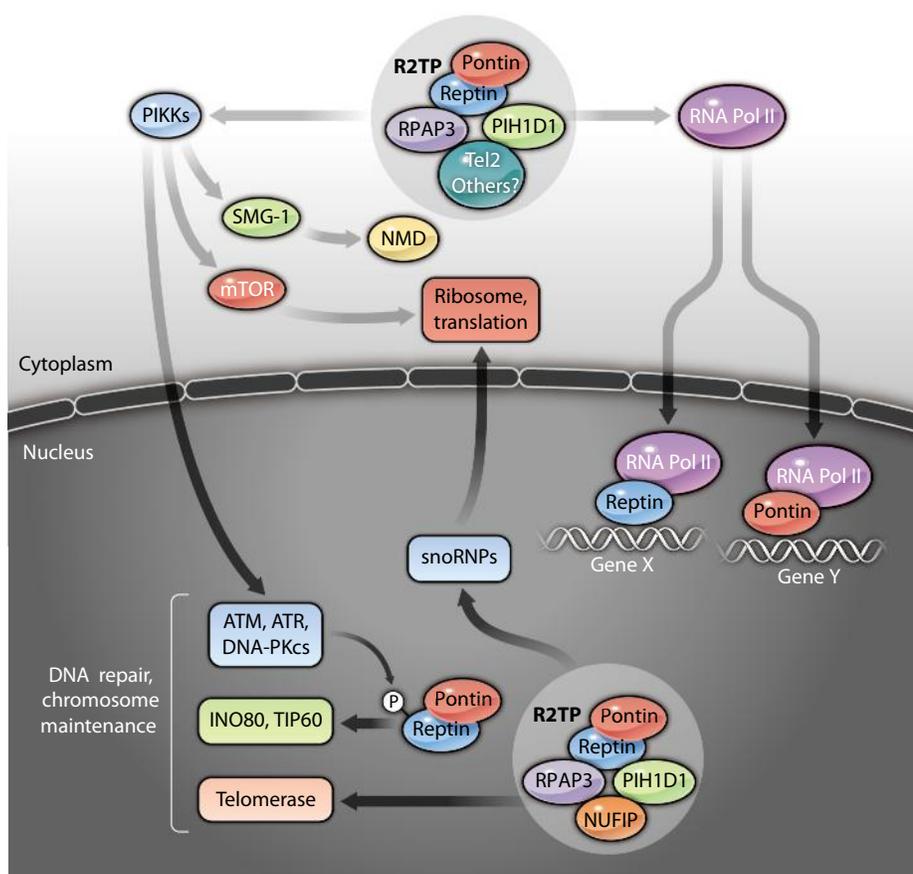


Fig. 3. Major functions of human Pontin and Reptin. In the cytoplasm, Pontin (red ovals) and Reptin (blue ovals) associate with RPAP3 and PIH1D1 to form the R2TP complex. This complex associates with the adaptor Tel2 and helps stabilize all members of the PIKK family, such as SMG-1 [which is involved in nonsense-mediated mRNA decay (NMD)]; mTOR (which regulates translation); and ATM, ATR, and DNA-PKcs (which are involved in DNA damage repair). Likely cooperating with another unidentified adaptor, the R2TP complex assembles RNA polymerase II (RNA Pol II) in the cytosol. To participate in the biogenesis of snoRNPs and telomerase, the nuclear R2TP complex associates with the adaptor nuclear fragile X mental retardation-interacting protein 1 (NUFIP) instead of Tel2 (30). In the nucleus, Pontin and Reptin are also involved in the assembly of INO80 and NuA4-TIP60 complexes, but it is unknown whether additional partners are required. A large-scale study has shown that Reptin is phosphorylated (P) on an ATM/ATR consensus site after DNA damage (46); thus far, the functional consequences are unknown. Pontin and Reptin also regulate gene transcription in association with RNA Pol II. Transcriptional regulation is the only context in which Pontin and Reptin appear to function separately.

explore the roles of these proteins and why this parasite requires three such proteins.

Conclusions

This First International Workshop on Pontin (RUVBL1) and Reptin (RUVBL2) has met its objectives, allowing researchers working independently on these proteins to meet and exchange ideas across diverse disciplines in the life sciences. The emerging view is that the diverse and largely overlapping functions of these two closely related proteins result from a highly diverse set of

protein-protein interactions that are likely controlled, in part, by the oligomeric nature of Pontin and Reptin and the possibility that these proteins act as molecular scaffolds that regulate the assembly and disassembly of protein complexes (Fig. 3). The diverse nature of these protein-protein interactions and their rewiring in pathophysiological states may impact the development of diseases such as cancer. Still, many issues remain unsettled, leaving ample room for discussions in the next meeting to be held in Portugal in 2014.

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