Integrated regulation of PIKK-mediated stress responses by AAA+ proteins RUVBL1 and RUVBL2

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Proteins of the phosphatidylinositol 3-kinase-related protein kinase (PIKK) family are activated by various cellular stresses, including DNA damage, premature termination codon and nutritional status, and induce appropriate cellular responses. The importance of PIKK functions in the maintenance of genome integrity, accurate gene expression and the proper control of cell growth/proliferation is established. Recently, ATPase associated diverse cellular activities (AAA+) proteins RUVBL1 and RUVBL2 (RUVBL1/2) have been shown to be common regulators of PIKKs. The RUVBL1/2 complex regulates PIKK-mediated stress responses through physical interactions with PIKKs and by controlling PIKK mRNA levels. In this review, the functions of PIKKs in stress responses are outlined and the physiological significance of the integrated regulation of PIKKs by the RUVBL1/2 complex is presented. We also discuss a putative "PIKK regulatory chaperone complex" including other PIKK regulators, Hsp90 and the Tel2 complex.

Introduction

Genome maintenance and precise gene expression are critically important issues for all organisms. Cells have evolved defense systems from gene mutations, errors in gene expression and various environmental stresses. Phosphatidylinositol 3-kinase-related protein kinase (PIKK) family proteins engage with these defense systems at each level of gene expression. Six PIKKs, ATM (ataxia telangiectasia mutated), ATR (ATM- and Rad3-related),

DNA-PKcs (DNA-dependent protein kinase catalytic subunit), SMG-1 (suppressor with morphogenetic effect on genitalia-1), TOR (target of rapamycin) and TRRAP (transformation/ transcription domain associated protein), have been identified in vertebrates. All PIKKs, except for TRRAP, function as protein kinases and transduce cellular stresses as phosphorylation signals to downstream effectors and induce proper stress responses. In addition to the importance of each PIKK function, recent studies have suggested an interplay among PIKKs. In this review, we provide an overview of the functions of PIKKs and present recent findings of common regulators of PIKKs. We also discuss a possible role of common regulators of PIKKs in the coordination of PIKKs in cellular stress responses.

PIKK-Mediated Defense Systems Against Various Cellular Stresses

PIKK family. The phosphatidylinositol 3-kinase-related protein kinase (PIKK) family is known as an atypical Ser/Thr protein kinase family that has sequence homology to the catalytic domain of lipid PI3-kinases.[1] These kinases are characterized as large proteins (270−470 kDa) with shared domain structures: a highly conserved catalytic domain, FAT-C (FRAP, ATM and TRRAP C-terminal) and successive α-helical repeats in the N-terminal region that provides protein-protein interaction surfaces (**Fig. 1**). Among the six PIKKs reported, ATM, ATR, TRRAP and TOR are evolutionarily conserved from *Saccharomyces cerevisiae* to *Homo sapiens*, whereas DNA-PKcs and SMG-1 appeared during metazoan evolution. ATM, ATR, DNA-PKcs and SMG-1 preferentially phosphorylate Ser or Thr followed by Glu; therefore, these proteins are called S/TQ directed kinases.[2] Every PIKK forms a protein complex with specific binding partners that can regulate the recruitment of PIKK to the activation site, substrate

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binding and kinase activity.[3] The PIKK complexes play central roles in cellular responses to various stresses, including DNA damage, aberrant mRNAs and nutrient availability (**Fig. 2**).

ATM (reviewed in refs. 4 and 5). ATM functions in responses to DNA double-strand breaks (DSBs), which are formed by ionizing radiation (IR) and DNA damaging agents. When DSBs appear, ATM is recruited to the adjacent region of the DSBs and is partially activated by autophosphorylation that transforms an inactive dimer to active monomers. In this early stage, ATM phosphorylates substrates including histone H2AX and p53. Phosphorylated histone H2AX becomes an initial signal for DNA damage and recruits DNA damage recognition/repair factors. Phosphorylated p53 induces the G_1 checkpoint. Monomer ATM is recruited to DSBs by the Mre11-Rad50-Nbs1 (MRN) complex and is fully activated. Active ATM phosphorylates diverse downstream effectors and DNA break associated proteins, including Chk2, Nbs1, MDC1, BRCA1 and induces cell-cycle checkpoint, DNA repair and stress-induced transcription.

Besides DNA damage responses, ATM is involved in vesicle transport in the cytoplasm. For example, ATM associates with β-adaptin, one of the components of the clathrin-mediated endocytosis adaptor complex.[6] Cytoplasmic vesicular localization of ATM, including peroxisome, is also observed and ATM deficient cells show increased lysosomal accumulation and reduced oxidative metabolism.[7,8] The cytoplasmic localization of ATM is especially appreciable in neural cells and ATM forms a complex with VAMP2 and Synapsin-I, two synaptic vesicle proteins, and modulates synaptic function through the regulation of the synaptic vesicular release cooperatively with ATR.[9] ATM also participates in insulin signaling by phosphorylating 4EBP1, a cap dependent negative translation regulator, collaborating with mTOR.[10]

Mutations of the ATM gene are responsible for ataxia telangiectasia (A-T), an autosomal recessive disorder characterized by cerebellar ataxia telangiectasia, immunodeficiency, radiosensitivity and cancer susceptibility.[11,12] ATM deficient mice show growth retardation, immunedefects, infertility, neurological defects and the majority of the mice develop thymic lymphomas.[7,13] ATM depletion also impairs stem cell maintenance and causes aged phenotypes.[14,15]

ATR (reviewed in ref. 16). ATR was originally discovered as a gene with sequence homology to ATM and is biochemically similar to ATM. In contrast to ATM, ATR is activated by a stalled replication fork during S phase and many types of DNA damage that give rise to single strand DNA (ssDNA) structures, including DSBs, base-crosslinks and agents, which cause DNA replication stress and DNA damage. ATR is recruited to the ssDNA coated with replication protein A (RPA) through the interaction with ATRIP. RPA also localizes the RAD9-RAD1-Hus1 (9–1–1) complex to the RPA-ssDNA sites. The 9–1–1 complex recruits TopBP1, an ATR activator, to ATR and induces ATR activation. Although ATR phosphorylates numerous substrates and regulates DNA replication, the cell cycle checkpoint and DNA repair, the best studied ATR substrate is Chk1. Activated Chk1 phospho-inactivates Ccd25 proteins, Cdk activators, thereby preventing the cell cycle transition. ATR-mediated Chk1 signaling is also critical for regulating DNA replication. ATR also phosphorylates replication related proteins, including PCNA, Polε, RPA, MCM proteins and DNA repair related proteins, including BRCA1, WRN and BLM. However, the physiological significance of these phosphorylation events is poorly understood. The kinase activity of ATR is also involved in replication-dependent histone mRNA degradation together with Upf1, a NMD transacting factor.[17]

As expected in the critical regulation of replication stress, ATR is essential for viability across many organisms ranging from yeast to mammals.[18,19] Moreover, deletion of the ATR gene in adult mice causes aged phenotypes and stem cell loss, in a similar manner to the ATM gene deletion.[20] Mutations of the ATR gene
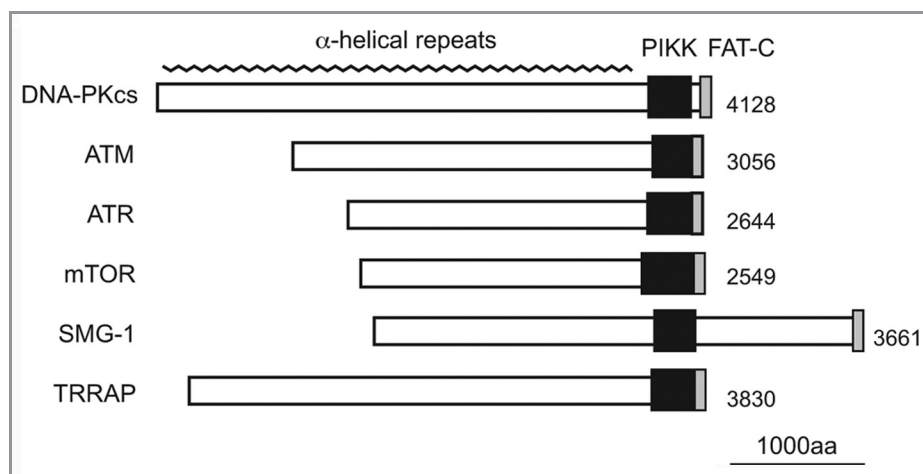
Figure 1. The domain structures of human PIKK family members. PIKKs share the highly conserved catalytic PIKK domain and the FAT-C (FRAP, ATM, and TRRAP C-terminal) domain. Although the PIKK domain has sequence homology to the catalytic domain of PI3-kinases, PIKKs act as Ser/Thr protein kinases except for TRRAP. The FAT-C domain located near the PIKK domain is thought to modulate the kinase activity. The N-terminal region of PIKK is composed of α-helical repeats, which contribute to protein-protein interactions.

Figure 2. Summary of PIKK-mediated stress responses. PIKKs are activated various cellular stresses and induce proper cellular responses at various steps of gene expressio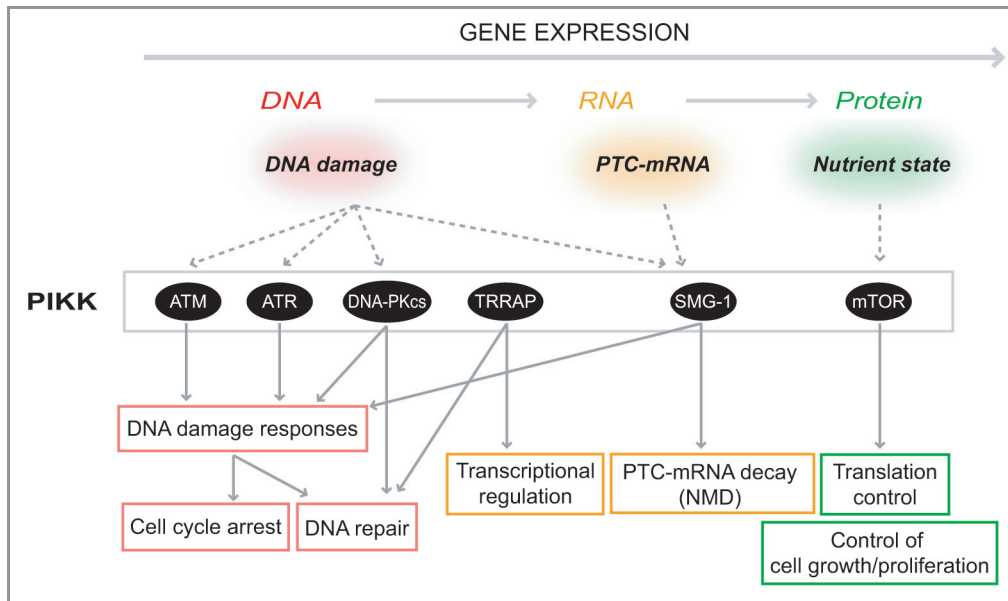n. ATM and ATR are activated by DNA damages including DSBs to arrest cell cycle and activate DNA repair pathways. DNA-PKcs engages in a DSB repair process called NHEJ. TRRAP regulates transcription as a HAT complex component. SMG-1 recognizes PTC-mRNAs and leads to PTC-mRNA degradation. mTOR controls cellular translation activity and cell growth in response to nutrient status. Except for TRRAP, each PIKK induces proper stress responses through phosphorylations of downstream effector proteins.

have been found in a few patients of the Seckel syndrome, an autosomal recessive disorder characterized by intrauterine growth retardation, microcephaly and mental retardation.[21]

DNA-PKcs (reviewed in refs. 22 and 23). DNA-PKcs is the catalytic subunit of the DNA-PK holoenzyme, which is composed of DNA-PKcs and the Ku70/80 heterodimer. DNA-PK (the DNA-PKcs and Ku70/80 complex) and its kinase activity are essential for non-homologous end-joining (NHEJ), a major DSB repair pathway in mammals. In NHEJ, Ku70/80 recognizes DNA ends and recruits DNA-PKcs to DSBs; thereby two DNA-PK molecules interact to connect the DNA ends. This interaction leads to the autophosphorylation of DNA-PK *in trans,* which induces conformational changes of DNA-PKcs and the release of the DNA-PKcs from the DNA ends, allowing the NHEJ- and repair factors to access the DSB and subsequent end-processing.[23] Besides autophoshorylation, a number of DNA-PK substrates including NHEJ factors have been identified in vitro. However, the physiological roles of these phosphorylation events in vivo have not been well defined. DNA-PK-mediated DNA end-processing is also required for the rejoining of DSBs generated by V(D)J recombination during T- and B-cell development, and a DNA-PKcs inactive mutation causes severe combined immuno-deficiency (SCID).[24,25] In addition to the role in NHEJ, recent studies have uncovered the involvement of DNA-PKcs in DNA damage signaling. For example, in response to IR, DNA-PKcs promotes cell survival through phosphorylation of Thr308 and Ser473 (this site is also phosphorylated by mTORC2, see below) residues of Akt (also called PKB) together with PDK1 and the subsequent transcriptional regulation of the p53-p21 pathway.[26] IR-dependent phospho-activation of nuclear caspase-2

by DNA-PKcs also contributes to NHEJ and the maintenance of the ATM-mediated G_2/M checkpoint.[27] Parts of DNA-PK are localized to lipid rafts, microenvironments on cell membranes where signaling molecules accumulate, and such localization has been suggested to mediate DNA damage signals through phosphorylations in response to IR.[28] In addition, DNA-PKcs mediates exchange of UV-induced translation profiles, including the promotion of the synthesis of DNA-repair related proteins and the inhibition of global translation.[29] DNA-PKcs is also involved in replication stress induced histone mRNA destabilization together with Upf1, similarly to that observed for ATR.[30] Furthermore, DNA-PK associates with telomeres and DNA-PK defects induce telomere fusion and telomere aneuploidy without telomere shortening, suggesting DNA-PK's critical role in telomere capping.[31]

SMG-1 (reviewed in refs. 32 and 33). SMG-1 forms an SMG-1 complex (SMG1C) with SMG-8 and SMG-9[34] and functions in an mRNA quality control mechanism called nonsense-mediated mRNA decay (NMD). NMD selectively degrades premature termination codon (PTC)-containing mRNAs, which can be generated by gene mutation, splicing and transcription errors. PTC-mRNAs also arise in a physiological process, the V(D)J recombination during T- and B-cell maturation.[35] Therefore NMD suppresses the production of potentially harmful or non-functional polypeptides and ensures the accuracy of gene expression. SMG-1 plays an essential role in NMD by phosphorylating Upf1, a central regulator of NMD. When a ribosome recognizes a PTC, SMG-1, Upf1 and eukaryotic releasing factors (eRF1 and eRF3) assemble to form the SMG-1-Upf1-eRF1-eRF3 (SURF) complex on the PTC-recognizing ribosome.[36] If an exon junction

complex (EJC), a multiprotein complex deposited on an exon-junction in a splicing dependent manner, exists downstream of the PTC, the SURF associates with the EJC. The association between SURF and EJC establishes PTC recognition and induces SMG-1-mediated Upf1 phosphorylation.[36] Phosphorylated Upf1 recruits mRNA decay factors and phopho-Upf1 recognizing NMD factors,[37-39] and advances subsequent decay processes. Therefore SMG-1-mediated Upf1 phosphorylation is an essential step in NMD. Although Upf1 is also identified as a substrate of other PIKKs (ATM, ATR, DNA-PKcs, see below), the function of SMG-1 in NMD cannot be compensated with other PIKKs.

In addition to NMD, SMG-1 is implicated in other stress responses, including DNA damage,[40] oxidative stress, hypoxia[41,42] and cytokine signaling.[43] In a similar fashion to ATM and ATR, SMG-1 activates by IR or UV and phosphorylates p53.[40] Moreover, SMG-1 depletion causes spontaneous DNA damage and sensitizes cells to IR.[40] SMG-1 also associates with the telomere and protects the telomere by inhibiting the association of telomeric repeat-containing RNA (TERRA) with telomeric DNA.[44]

SMG-1 is essential for mouse embryogenesis.[45] SMG-1 null mutants in *C. elegans* and *D. melanogaster* are viable,[46,47] and inactivation of SMG-1 shows oxidative stress resistance and longevity in analogy to TOR in *C. elegans*.[48] Since NMD suppresses the dominant phenotype of the heterozygote caused by a nonsense mutation and because NMD is not essential for viability in *C. elegans*, a temperature sensitive mutant of SMG-1 can be used for genetic screening to identify gene mutations in heterozygotes of *C. elegans*. Temperature sensitive mutants of SMG-1 have also been used for inducible expression of transgenes with long 3'UTRs, which are a NMD target.[49]

mTOR (reviewed in ref. 50). TOR was originally identified as the target protein of rapamycin, a macrolide produced by bacterium *Streptomyces hygroscopicus*.[51,52] TOR regulates various cellular activities, including cell size control, cell proliferation, translation activity and cell metabolism in response to external stresses and nutritional status. From yeast to mammals, two distinct functional TOR complexes have been identified: TORC1 and TORC2. Mammalian TORC1 (mTORC1), which is directly inhibited by rapamycin, is composed of mTOR, Raptor and mLST8 (also called as GβL), whereas rapamycin insensitive mTORC2 is composed of mTOR, Rictor, mLST8, SIN1 and Protor.

mTORC1 functions as a sensor of external signals, such as growth factors, nutrients, redox stress and controls cellular translation activity.[53] The mTORC1 phosphorylates the p70 ribosomal S6 kinase (S6K) and eIF4E binding protein (4EBP), two key regulators of cap-dependent translation, thereby facilitating translation together with the regulation of ribosome biogenesis via transcriptional regulation.[54] mTORC1 also enhances the translation efficiency of newly synthesized spliced mRNAs through activation of S6K recruited to the spliced mRNAs by SKAR, an EJC component.[55] mTORC1-mediated S6K phosphorylation and translation enhancement are linked to cell size control.[56] mTORC1 also acts as a conserved negative regulator of autophagy in response to nutrient availability.[57]

In contrast, mTORC2 regulates actin cytoskeletal organization by phosphorylating PKCα[58,59]; however, the upstream signals remain unclear. mTORC2 also phosphorylates Ser473 of Akt and controls cell growth, proliferation and cell migration.[60] Recently, another (m)TORC2 target, serum glucocorticoid–induced protein kinase 1 (SGK1) has been identified.[61] Through the phosphorylation of SGK1, TORC2 regulates fat metabolism, body size and development in *C. elegans*.[62,63]

Based on the critical role of mTOR in regulating cell proliferation and growth, mTOR knockout mice are embryonic lethal and mTOR-deficient embryonic stem (ES) cells fail to be maintained because of proliferation arrest.[64,65] Moreover, (m)TORC1 signaling is linked to aging and the selective inhibition of TORC1 signaling commonly extends the life span of yeast, worms, flies and mice.[66]

TRRAP (reviewed in ref. 67). TRRAP, the only catalytically inactive PIKK member, was identified as an essential co-factor of c-Myc and E2F for the transcription/transformation activities of these oncogenic proteins and named "transformation/transcription domain–associated protein (TRRAP)"[68]. Beside c-Myc and E2F, TRRAP associates with various transcription factors, including p53, E1A, ERα/β, β-catenin and regulates transcription. Importantly, TRRAP is a shared and essential component of distinct histone acetyltransferase (HAT) complexes, including PCAF (SAGA in *S. cerevisiae*), TIP60 (NuA4 in *S. cerevisiae*), TFTC and SILK HAT complexes from yeast to mammals. A HAT complex, which is composed of a catalytic subunit and its associated proteins, acetylates Lys residues of core histone tails to promote transcription. TRRAP appears to play a role in the recruitment of HAT complexes to the chromatin as a mediator between a HAT complex and various transcription factors. A genome-wide analysis revealed that TRRAP regulates various gene expressions involved in cell cycle progression, the cytoskeleton, cell adhesion, protein turnover, metabolism and signal transduction.[69] Consistent with this, TRRAP is essential for viability in *S. cerevisiae* and *Mus musculus*,[70,71] and TRRAP-deficient cells show mitotic checkpoint failures and the severe suppression of cell proliferation.[70] TRRAP is also involved in DNA repair processes through the TIP60 HAT complex[72] and the NHEJ pathway.[73]

RUVBL1 and RUVBL2 are Multifunctional ATPases (reviewed in ref. 74)

RUVBL1 and RUVBL2 are conserved AAA+ family proteins. RuvB-like (RUVBL) 1 and RUVBL2, also known as Pontin and Reptin (or TIP48 and TIP49), are evolutionarily conserved ATPases that belong to the ATPase associated diverse cellular activities (AAA+) family. They have sequence homology to bacterial RuvB, a DNA helicase involved in homologs recombination and DNA repair.[75,76] Both have been shown to have ATPase and DNA helicase activity in vitro.[77] RUVBL1 and RUVBL2 interact with each other and can form a double hexamer, probably consisting of two homo hexamers, which is a characteristic of AAA+ family proteins.[78,79] In some circumstances, RUVBL1 and RUVBL2 act independently and have antagonistic

effects.[80,81] However, in most cases, they appear to form a complex and function together. The complex formation of RUVBL1 and RUVBL2 appears to be important in vivo, because the depletion of either protein causes co-depletion of the other protein.[82,83] Based on their diverse functions (**Fig. 3**), both proteins are essential for viability/development of *S. cerevisiae*,[77,85] *D. melanogaster*,[86] and *C. elegans* [wormbase (http://www.wormbase.org/)].

The RUVBL1/2 complex is involved in diverse cellular functions. While RUVBL1 and RUVBL2 participate in diverse nuclear processes, the best studied function of RUVBL1 and RUVBL2 is transcriptional control.[87] RUVBL1 and RUVBL2 are shared components of the Ino80 and SRCAP (Swr1 in *S. cerevisiae*) chromatin remodeling complex,[88,89] and the TIP60 HAT complex.[90] The RUVBL1/2 complex is essential for the chromatin remodeling activity of the Ino80 complex[91] and the HAT activity of the TIP60 complex.[92] Both the Ino80 and TIP60 chromatin remodeling/modifying complexes are also implicated in DNA repair.[93,94] Moreover, RUVBL1 and RUVBL2 interact with RNA polymerases and various transcription factors, including c-Myc, β-catenin, TATA binding protein and E2F, and regulate transcription of their target genes, thereby affecting cellular transformation, development and tumor metastasis.[87] Conditional depletion of *Rvb1* or *Rvb2* in *S. cerevisiae* was reported to influence transcription of > 5% of the genes of yeast.[95]

The RUVBL1/2 complex is also involved in the maturation of small nucleolar RNPs (snoRNPs), which catalyze post-transcriptional modification of non-coding RNAs, such as rRNA (rRNA), tRNA (tRNA) and small nuclear RNA (snRNA).[96] The RUVBL1/2 complex associates with different snoRNPs and controls accumulation of snoRNAs, proper localization of the components to the nucleolus and snoRNP assembly.[97,98]

The RUVBL1/2 complex also participates in telomere maintenance. The RUVBL1/2 complex associates with telomerase reverse transcriptase (TERT) and regulates the accumulation of the telomerase RNA component (TERC) and the assembly of the functional telomerase complex with its ATPase activity. Therefore, depletion of RUVBL1 or RUVBL2 severely reduces telomerase activity.[83] The association of the RUVBL1/2 complex with TERT is observed to peak in the S phase,[83] suggesting that the RUVBL1/2 complex regulates telomerase activity in a cell cycle dependent manner.

In addition, the RUVBL1/2 complex was identified as one of interacting proteins of Hsp90, an essential molecular chaperone for cellular homeostasis, together with RPAP3 and NOP17 (Tah1 and Pih1 in *S. cerevisiae*, respectively), two conserved Hsp90 co-factors[99,100] (described later, see Section 4). The RUVBL1/2 complex was also found in another chaperone-like URI/prefoldin complex, which is involved in (m)Tor-dependent nutrient signaling[101] (described later, see Section 4).

RUVBL1 and RUVBL2 are also involved in mitosis. RUVBL1 interacts with α- and γ-tubulin, and regulates spindle assembly,[102] whereas RUVBL2 is localized to the midbody and suggested to function in cytokinesis.[103,104]

As mentioned above, the RUVBL1/2 complex participates in various cellular processes. In most cases, the RUVBL1/2 complex
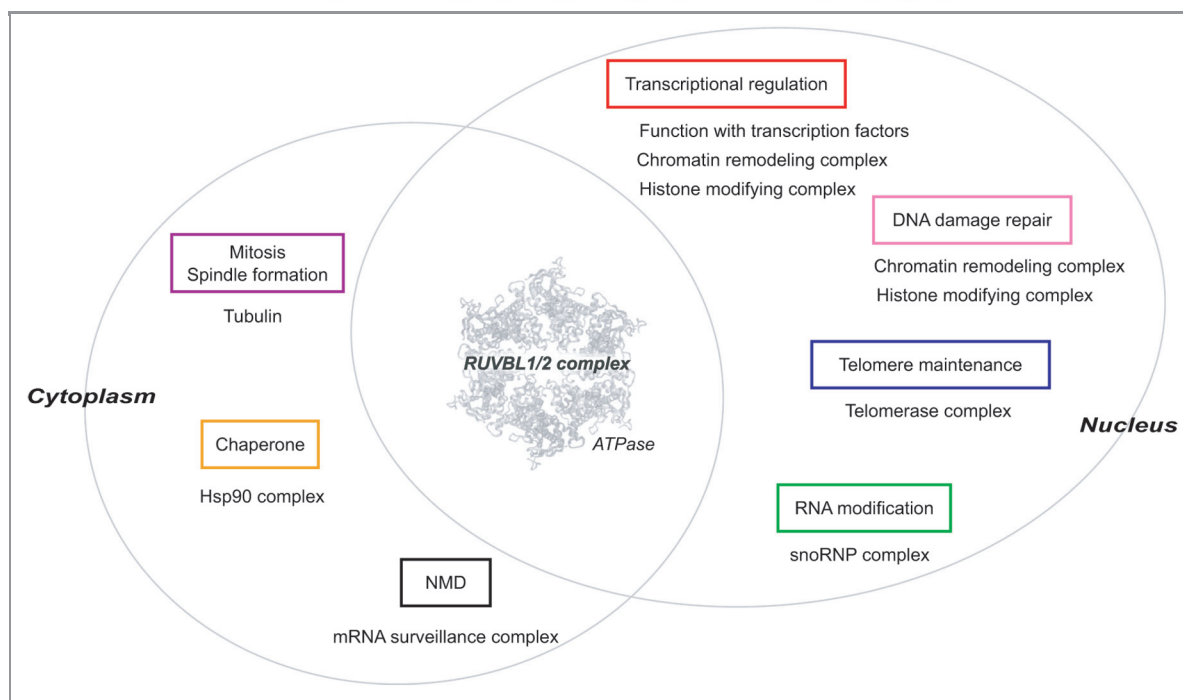

Figure 3. The RUVBL1/2 complex participates in diverse cellular processes. The RUVBL1/2 complex is composed of RUVBL1 and RUVBL2, and both proteins possess ATPase activity. The RUVBL1/2 complex is localized to nucleus and cytoplasm, and participates in diverse cellular processes together with specific interactors (shown below each box). The ATPase activity of the RUVBL1/2 complex is thought to essential for their functions in each process. The atomic structure of RUVBL1 is derived from reference 84.

functions as a component of multiprotein complexes containing nucleic acids (DNA/RNA), and it commonly regulates the assembly of these functional complexes.[82,83,91] Inhibition of the RUVBL1/2 complex causes immature complex assembly and functional defects, suggesting a similar mode of action of the RUVBL1/2 complex on macromolecular complex formation/ remodeling. Although the detailed mechanisms are unknown, their ATPase activity and nucleic binding properties may be important for these processes.

Integrated Regulation of the PIKK Family by the RUVBL1/2 Complex

The RUVBL1/2 complex regulates PIKK function through physical interaction and controls the levels of these kinases. Recently, we found an unexpected link between the RUVBL1/2 complex and the PIKK family. We had originally identified RUVBL1 and RUVBL2 as SMG-1 interacting proteins. Subsequent analyses revealed that the RUVBL1/2 complex associates not only with SMG-1 but also with any PIKK.[82] In addition to the physical interactions, the RUVBL1/2 complex regulates the levels of all PIKKs (**Fig. 4A**). Either knockdown of RUVBL1 or RUVBL2 clearly decreased all PIKK proteins and suppressed PIKK signaling.[82] Thus, the RUVBL1/2 complex can modulate PIKK functions as a common interactor and regulator of their protein abundance.

The detailed mechanism describing how the RUVBL1/2 complex controls the quantities of PIKKs is unknown; however, regulation appears to be at the mRNA level and the ATPase activities of both RUVBL1 and RUVBL2 are involved.[82] As one possibility, the RUVBL1/2 complex may regulate transcriptional activity of PIKKs together with E2F1 and c-Myc, because E2F1, one of RUVBL1 interacting transcription factors and regulated by c-Myc, can promote transcriptional activity of ATM and DNA-PKcs.[106,107] E2F1 and c-Myc also facilitate translation activity of target mRNAs by inducing cap methylation;[108] therefore, the RUVBL1/2 complex may influence the translation activity of PIKK mRNAs. Actually, the effect of RUVBL1/2 knockdown on the PIKK protein levels is more severe than that on the PIKK mRNA levels,[82] indicating that an undefined mechanism at the protein level participates in the process. Given the association of the RUVBL1/2 complex with Hsp90 and the Tel2 complex, the RUVBL1/2 complex probably acts through the Hsp90 chaperone pathway for maturation and stabilization of PIKK proteins (**Fig. 4A**, described later, see Putative "PIKK Regulatory Chaperone Complexes" Consisting of the RUVBL1/2 Complex, the Tel2 Complex and HSP90).

As described above, the RUVBL1/2 complex directly participates in the functions of at least two PIKKs, TRRAP and SMG-1. TRRAP and the RUVBL1/2 complex function together in transcriptional regulation and DNA repair processes as essential components of the TIP60 HAT complex.[72,87,90] On the other hand, the RUVBL1/2 complex associates with SMG-1 and facilitates rearrangement of the SMG-1-containing complex during NMD.[82] Since RUVBL1 and RUVBL2 interact with the N-terminal region of SMG-1,[82] the RUVBL1/2 complex

is expected to interact with α-helical repeats of other PIKKs (**Fig. 1**). The α-helical region of PIKKs provides protein-protein interaction surfaces important for their functions, such as ATM-Nbs1, ATR-ATRIP, mTOR-Raptor and SMG-1-SMG-8/SMG-9;[109-112] therefore the association of the RUVBL1/2 complex possibly influences the formation of PIKKs complexes.

In a different manner from TRRAP and SMG-1, a direct relationship between the RUVBL1/2 complex and other PIKKs has not been reported. However, previous studies suggest the involvement of the RUVBL1/2 complex in PIKK-mediated DNA damage response and repair. For example, the RUVBL1/2 complex-containing the TIP60 HAT complex acetylates the FAT-C domain of ATM, thereby activating ATM in response to DNA damage.[113] The requirement of the RUVBL1/2 complex for the TIP60 HAT activity[92] indicates a critical role of the RUVBL1/2 complex in ATM activation and the DNA damage response. The FAT-C domain is conserved among PIKKs and critical for kinase activity (**Fig. 1**);[114–117] therefore other PIKKs may be activated by similar acetylation events.[118] The RUVBL1/2 complex may also be involved in ATR recruitment through physical interactions with RPA3,[85] a subunit of RPA, an ATR recruiter. Moreover, RUVBL2 is a DNA damage-induced ATM/ATR substrate.[105] These observations indicate that the RUVBL1/2 complex directly participates in the PIKK-mediated DNA damage response and repair process in addition to the quantity control of PIKKs (**Fig. 4B and C**).

Although ATM, ATR and DNA-PKcs have been established as nuclear kinases, the RUVBL1/2 complex associates with PIKKs both in the nucleus and cytoplasm (unpublished data), suggesting that the RUVBL1/2 complex may also influence the nuclear localization of PIKKs or their cytoplasmic functions (see Section 1). For instance, a part of ATM, ATR and DNA-PKcs localizes to the centrosome[119] and ATM/ATR activates the cell cycle checkpoint by inhibiting spindle assembly in response to DNA damage during mitosis.[120] As mentioned above, the RUVBL1/2 complex associates with α- and γ-tubulin[103,121] and RUVBL1 regulates microtubule assembly during mitosis,[102] implying a relationship to the ATM/ATR-mediated DNA damage response during mitosis.

Functional relationships between the RUVBL1/2 complex and TOR have also been suggested. The (m)TORC1 acts as a positive regulator of transcription of rRNAs and ribosomal proteins.[54] In addition, TORC1 controls rRNA maturation through snoRNP localization/accumulation in the nucleolus like RUVBL1 in *C. elegans*,[122] suggesting that TOR and RUVBL1 function in the same pathway. A further study indicated that the RUVBL1/2 complex participates in (m)TOR signaling as components of the unconventional prefoldin URI complex together with RPB5[101] (described later, see Putative "PIKK Regulatory Chaperone Complexes" Consisting of the RUVBL1/2 Complex, the Tel2 Complex and HSP90).

Taken together, the RUVBL1/2 complex can regulate PIKK functions thorough several ways: (1) control of PIKKs levels (**Fig. 4A**); (2) activation of PIKKs via post translational modifications (**Fig. 4B**); (3) recruitment or localization of PIKKs; (4) promote assembly/rearrangement of PIKK complexes (**Fig. 4B**);
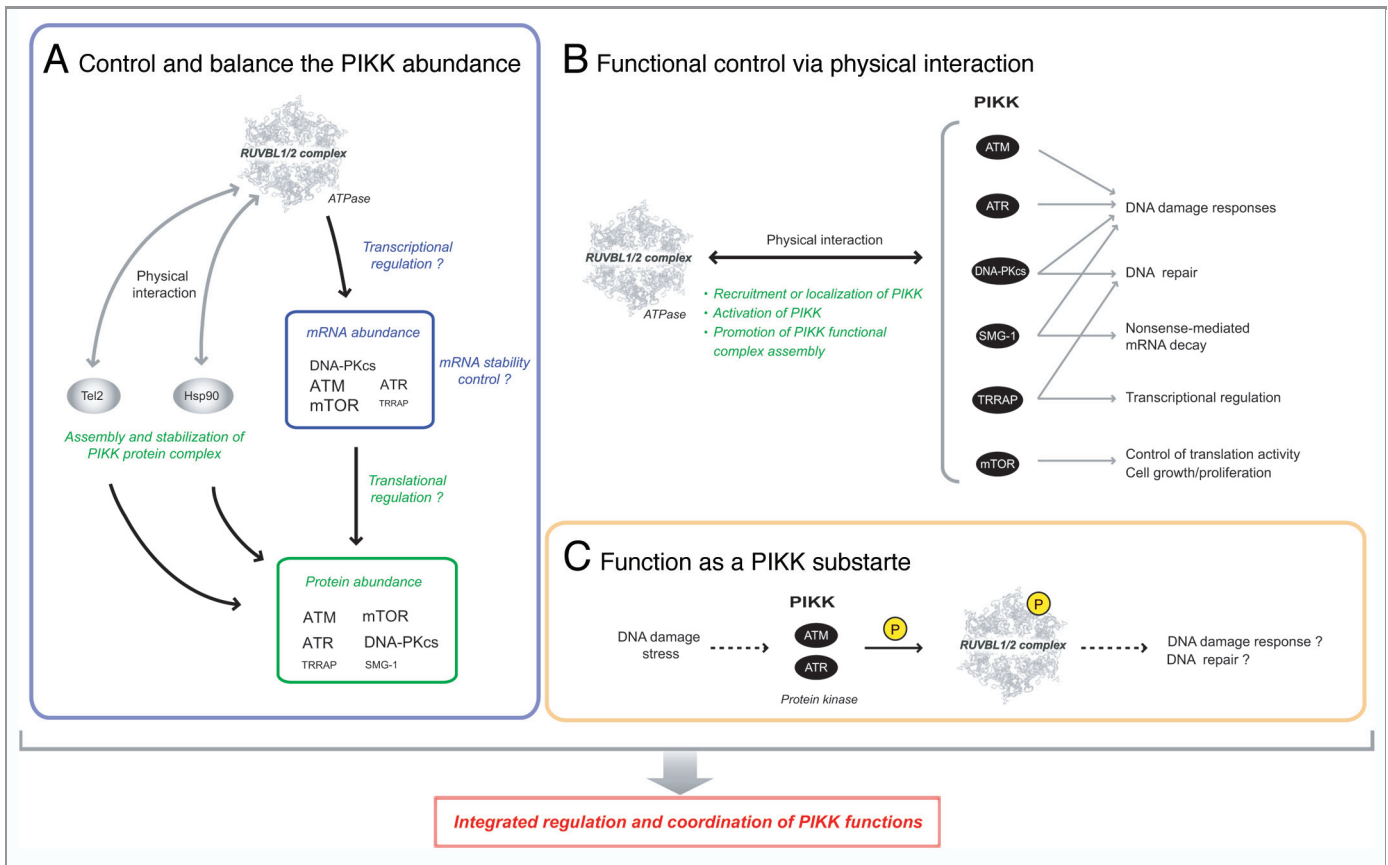
Figure 4. The RUVBL1/2 complex can regulate PIKK functions through several ways. Three possible mechanisms for the RUVBL1/2 complex to regulate PIKK functions. (A) Control and balance the abundance of PIKK. The RUVBL1/2 complex and its ATPase activity is required for the maintenance of PIKK protein abundance. The RUVBL1/2 complex affects the mRNA level of some PIKKs. The character size of each PIKK shows the extent of the sensitivity. The RUVBL1/2 complex is also involved in the assembly and stabilization of newly synthesized PIKK protein complex probably together with Hsp90 and the Tel2 complex. (B) Functional control via physical interactions. The RUVBL1/2 complex physically interacts with PIKK and facilitates proper PIKK-mediated stress responses. Three mechanisms to control PIKK function; recruitment/localization of PIKK, activation of PIKK through posttranslational modification, and promotion of the functional complex assembly of PIKK during stress responses. (C) Function as a PIKK substrate. RUVBL2 is phosphorylated by ATM/ATR in response to DNA damage stress.[105] The RUVBL1/2 complex may have a role as a downstream effector protein of PIKKs. The atomic structure of RUVBL1in **Figure 4** is derived from reference 84.

and (5) function as a downstream effector of PIKK signaling (**Fig. 4C**).

Functional links among PIKKs and a possible role of the RUVBL1/2 complex in the coordination of PIKK-mediated stress responses. What is the significance of common PIKK regulators? Based on previous observations suggesting functional links among PIKKs, the RUVBL1/2 complex may participate in coordinating and regulating PIKK signaling for the appropriate stress responses (Possible models are illustrated in **Figure 5A**).

Previous studies have often suggested functional relationships among PIKKs in DNA damage responses. For example, ATM and ATR are postulated to be activated by separate signals and act independently. However, interdependent activation between ATM and ATR is also observed [**Fig. 5B**-(a)].[123-125] In addition, ATM or ATR phosphorylates DNA-PKcs in response to IR or UV/replication stress and the former is important for cellular radio-resistance and NHEJ [**Fig. 5B**-(b)].[126,127] Conversely, DNA-PKcs regulates the abundance of ATM and SMG-1

[**Fig. 5B**-(c)].[128,129] SMG-1 also activates in response to IR and UV, and phosphorylates p53 together with ATM/ATR.[40] In DNA repair processes, TRRAP contributes to efficiency/fidelity of NHEJ in a HAT activity independent manner, in addition to DNA-PKcs.[73] ATM/ATR-mediated DNA damage signals link to various signal pathways. Upstream and downstream factors of mTORC1, Akt, TSC1, 4EBP and S6K have been identified as possible ATM/ATR substrates induced by IR[105] and down-regulation of mTORC1 signaling by DNA damage stress has been reported [**Fig. 5B**-(d)].[130] In contrast, mTOR regulates ATM levels [**Fig. 5B**-(c)].[128] In addition, (m)TORC1 inhibition and *tor1* (one of tor genes in *S. pombe* and forms TORC2) mutants show high sensitivity to DNA-damaging agents,[131-133] suggesting a link between ATM/ATR-mediated DNA damage responses and (m)TOR signaling. We speculate that PIKKs function in DNA damage response and DNA repair in collaboration with each other at multiple levels, and this is important for proper DNA damage responses. In this context, the RUVBL1/2 complex
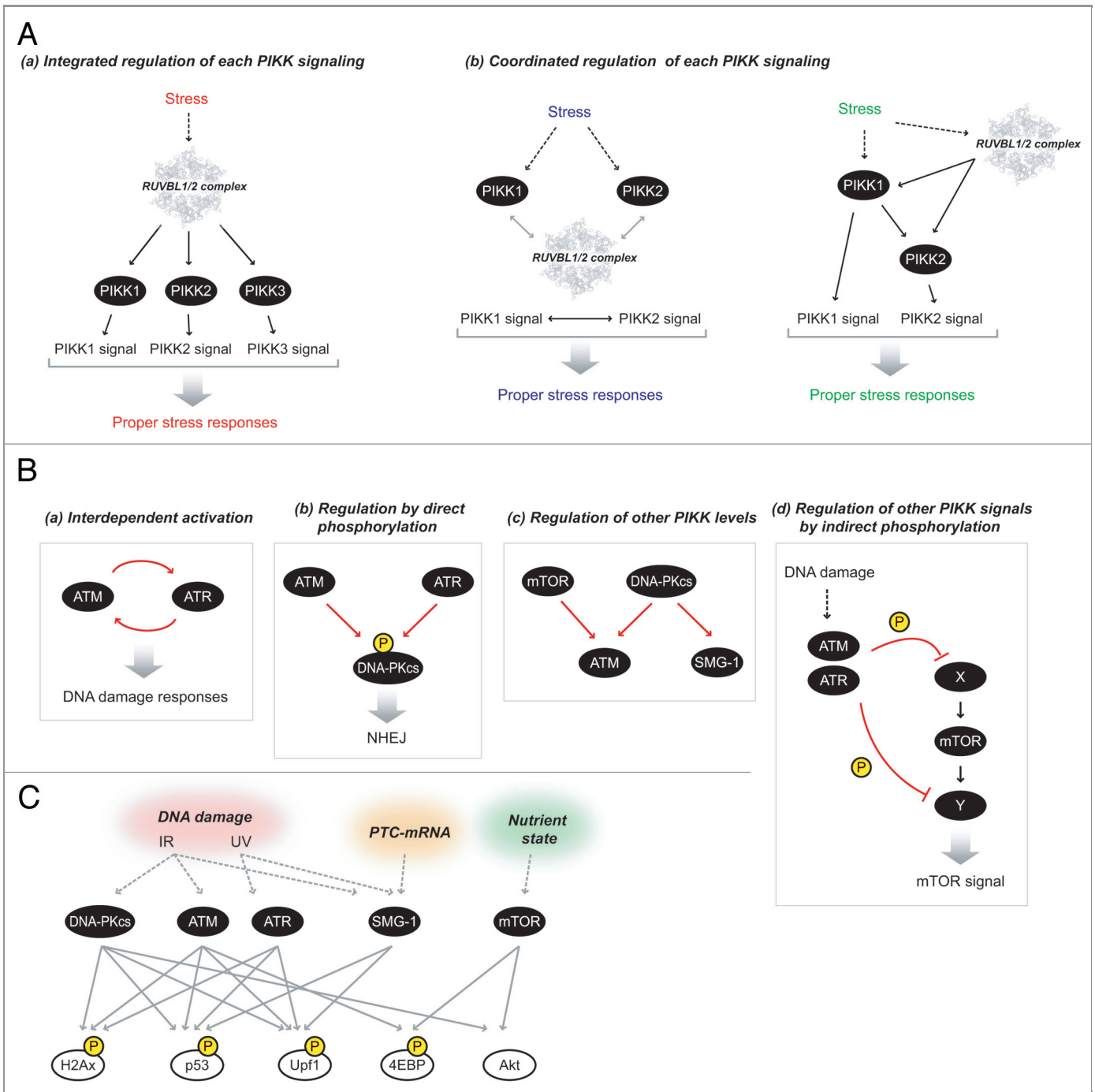
Figure 5. Crosstalk and regulation among PIKKs. (A) Possible models of the regulation of PIKK signaling by the RUVBL1/2 complex. (a) The RUVBL1/2 complex integrates each PIKK signaling as an upstream regulator and induces proper stress responses. (b) When multiple PIKKs cooperatively function in response to stress signals, the RUVBL1/2 complex assists this process and coordinates multiple PIKK signals (the left model). The RUVBL1/2 complex coordinates the cross-regulation among PIKKs [see also (B)] thereby induce proper stress responses (the right model). The atomic structure of RUVBL1 is derived from reference.[84] (B) Cross-regulation among PIKKs. Several regulatory mechanisms among PIKKs have been observed. (a) Interdependent activation of ATM and ATR in response to DNA damage. (b) Regulation of other PIKK by direct phosphorylation: DNA-PKcs is phosphorylated by ATM and ATR in response to DNA damage stress to regulate cellular radio-resistance and NHEJ. (c) Regulation of other PIKK levels: DNA-PKcs and mTOR are required for the maintenance of ATM abundance. DNA-PKcs is also involved in the maintenance of SMG-1 abundance. (d) Regulation of other PIKK signals by indirect phosphorylations: Both upstream and downstream factors of mTORC1 signal are ATM/ATR substrates and mTORC1 signal is downregulated by DNA damage stresses. (C) Shared substrates among PIKKs. Histone H2Ax, p53, and Upf1 are shared substrates of DNA-PKcs, ATM, ATR and SMG-1. 4EBP and Akt, two well known mTOR substrates, are also phosphorylated by ATM and DNA-PKcs respectively.

can serve as a mediator among PIKKs and organize DNA damage responses.

Another possible functional link among PIKKs is telomere maintenance. The telomere is a protective end structure of chromosomes in eukaryotes and is essential for genome stability.[134] The telomere is maintained by telomerase, an RNP complex containing the telomerase reverse transcriptase catalytic subunit (TERT), and protected by multiple telomeric DNA binding proteins. Telomere maintenance closely links to DNA damage repair processes[135] and at least four of the six PIKKs are involved in telomere maintenance. For example, Tel1 and Rad3 (ATM and ATM orthologs in *S. pombe*) promote the recruitment of telomere protective proteins and telomerase.[136] ATM and ATR also cooperate with other repair machinery to form the proper telomeric structure on telomere replication.[137] DNA-PKcs and Ku70/80 associate with telomeres and are suggested to function in telomere capping.[31] SMG-1 also associates with telomeres and inhibits accumulation of TERRA around the telomere and SMG-1 depletion causes telomere loss and fusion.[44] In most somatic cells, telomerase expression is low, while progenitor germ/stem cells and putative cancer stem cells possess high activity of telomerase. When a silent TERT gene reactivates, c-Myc, TRRAP and its associating HAT activities are required.[138] TRRAP-containing SAGA HAT complex also regulates the turnover of critical telomere binding protein, TRF1.[139] Several reports also suggest the involvement of mTOR in telomere regulation. For example, mTORC1 inhibition causes downregulation of TERT mRNA expression and reduced telomerase activity.[140] On the other hand, Akt, a downstream effector of mTORC2, negatively regulates telomere length by phosphorylating TRF1,[141] which is consistent with another study showing the elongation of the telomere in a *tor1* mutant in *S. pombe*.[131] As mentioned above, the RUVBL1/2 complex is critical for telomerase activity as this complex promotes the assembly of the telomerase complex.[83] Although the direct relationship among PIKKs and the RUVBL1/2 complex in telomere maintenance has not been defined, their cooperative actions and the coordination of PIKKs by the RUVBL1/2 complex may be important for telomere maintenance.

In addition to the above mentioned cases, several PIKK substrates, including p53, histone H2AX, Upf1, 4EBP and Akt are shared by multiple PIKKs (**Fig. 5C**). Thus, the RUVBL1/2 complex may be involved in the selection of PIKKs through a cellular stress dependent mechanism.

Putative "PIKK Regulatory Chaperone Complexes" Consisting of the RUVBL1/2 Complex, the Tel2 Complex and Hsp90

Two other PIKK regulators, the Tel2 complex and Hsp90. In addition to the RUVBL1/2 complex, at least two common regulators of PIKK, the Tel2 complex and Hsp90, have been reported.

Tel2 (also called CLK2) is the mammalian homolog of *S. cerevisiae* telomere maintenance 2 (Tel2); however, the involvement of Tel2 in telomere maintenance has not been reported in mammals.[142] Tel2 forms a complex with the Tel2-interacting protein 1 (Tti1) (also called SMG-10) and Tel2-interacting protein 2 (Tti2)[128,143-145]. Tel2, Tti1 and Tti2 are closely related and each protein level depends on one another.[128,143,144] The Tel2 complex associates with newly synthesized PIKK proteins and is required for the formation of PIKK-functional complexes, such as mTORC1, mTORC2 and ATR-ATRIP complexes.[144,146] Inhibition of the Tel2 complex causes destabilization of PIKK proteins without affecting their mRNA levels.[142-144] Moreover, Tel2 is required for the recruitment of Tel1 (ATM ortholog in *S. pombe*) to DNA damage sites and the activation of the Rad3 (ATR ortholog in *S. pombe*) and Rad3 mediated replication checkpoint.[147-150] Tti1 was also isolated as one of the genes required for IR-resistance and is involved in PIKK-mediated DNA damage responses.[143]

Hsp90 is an ATP-dependent molecular chaperone to specific proteins including various protein kinases. Hsp90 forms multiple complexes with its co-factors and facilitates structural maturation and complex assembly of its client proteins.[151] Although physical interactions of Hsp90 with PIKKs have been observed only in DNA-PKcs and SMG-1,[82,152] Hsp90 inhibition leads to a reduction in all PIKK proteins and their downstream phosphorylation signals.[82,128,142] Moreover, Hsp90 inhibition impairs DSB repair and the cell cycle checkpoint to IR because of the attenuation of IR-induced ATM and DNA-PKcs activation without downregulation of ATM and DNA-PKcs.[153] Hsp90 was also identified as a Raptor interacting protein and the reduction in phosphorylation of mTOR and its substrates, 4EBP and S6K, were observed under Hsp90 inhibition without affecting the levels of mTOR.[154]

The putative PIKK regulatory chaperone complex and its components. A recent report revealed that the Tel2 complex interacts with Hsp90 and that the associations between the Tel2 complex and PIKKs depends on the chaperone activity of Hsp90.[144] In addition, the RUVBL1/2 complex associates with the Tel2 complex and Hsp90.[82,128,144,166] Both the RUVBL1/2 complex and the Tel2 complex also interact with two evolutionarily conserved Hsp90 co-factors, RPAP3 and NOP17,[82,128,144] which can affect ATPase activity of Hsp90.[155,156] These observations strongly suggest that the RUVBL1/2 complex, the Tel2 complex and Hsp90 form a complex and function together to regulate PIKKs.

If it was the case, how do these molecules function together? One possibility is that the RUVBL1/2 complex and the Tel2 complex act as Hsp90 co-factors. Hsp90 cofactors play important roles in controlling Hsp90 chaperone activity through mediating client association and/or regulating the ATPase cycle of Hsp90.[151,157] Since Tel2 directly interacts with ATM and mTOR,[142] the Tel2 complex may promote the association of Hsp90 with PIKKs. Alternatively, the RUVBL1/2 complex and the Tel2 complex may affect the Hsp90 ATPase cycle, thereby regulating its chaperone activity. Considering that RUVBL1 and RUVBL2 are AAA+ family ATPases, which are widely involved in molecular remodeling events via ATP hydrolysis,[158] it is also possible that the RUVBL1/2 complex may act as another molecular chaperone in PIKK regulation. Indeed, the ability of

the RUVBL1/2 complex to maintain PIKK abundance requires its ATPase activity.[82] In this context, the associations between the RUVBL1/2 complex and Hsp90 cofactors (RPAP3, NOP17) may link two molecular chaperones like Hop, which mediates and coordinates two chaperones, Hsp70 and Hsp90.[159]

Interestingly, the RUVBL1/2 complex interacts with another chaperone-related prefoldin complex containing URI, RPB5 and Monad.[101,160] URI (also called RMP), an unconventional prefoldin, controls a part of nutrient sensitive gene expression and cell survival signaling downstream of (m)TOR,[101,161] and its deficiency causes DNA breaks and cell cycle arrest in *C. elegans*.[162] URI interacts with all PIKK proteins, the Tel2 complex, and Hsp90.[128] RPB5, a shared subunit of RNA polymerases and a known URI interactor,[163,164] associates with at least one PIKK, SMG-1, and is involved in NMD.[82] Monad (also called WDR92) interacts with at least the RUVBL1/2 complex, Tti1, RPAP3, NOP17, URI and RPB5.[82,128,160,165] Based on the above mentioned observations, multiple chaperone-containing complexes are expected to collaboratively function to regulate PIKKs (**Fig. 6**). Together with the previous analyses, the putative PIKK regulatory chaperone complex may not only assist the maturation of PIKK complexes when PIKK proteins are synthesized, but also facilitate the remodeling of PIKK complexes when PIKKs activate in response to stress signals. Interestingly, some molecules including RUVBL2 have putative phosphorylation sites by PIKK (see **Table 1**), suggesting that they can also function as PIKK downstream effectors and provide an additional intricate regulatory mechanism of PIKKs.

Given that the majority of the putative PIKK regulatory chaperone complex components also physically and functionally associate with transcriptional machinery[167,168] and RNP biogenesis,[169,170] similar complexes probably function in other cellular processes.

On the other hand, the inhibition of the RUVBL1/2 complex or the Tel2 complex has been observed to have a different effect on the PIKK mRNA levels.[82,142,143] Concerning the regulation of the PIKK abundance, the mutual regulation among PIKKs is also exist [**Fig. 5B**-(c)]. The regulatory mechanisms of the PIKK family appear to be involved in multiple unknown mechanisms. Further studies are required to understand the detailed molecular mechanisms of PIKK regulation by the putative PIKK regulatory chaperone complex.

Relationship of the RUVBL1/2 Complex to Cancer Biology

From a clinical point of view, PIKKs have been suggested to be potential therapeutic targets for cancer therapy. For example, the constitutive activation of mTOR signaling has been observed in multiple types of tumors, and rapamycin analogs, which inhibit mTORC1 and cause growth reduction of cancer cells, are under clinical trials as anti-cancer agents.[171] Further, ATM, ATR or DNA-PKcs-mediated DNA damage responses and DNA repair pathways are potential targets for cancer therapy in combination with irradiation and DNA-damaging chemical agents.[172] NMD inhibition is also attractive as a new therapeutic approach to
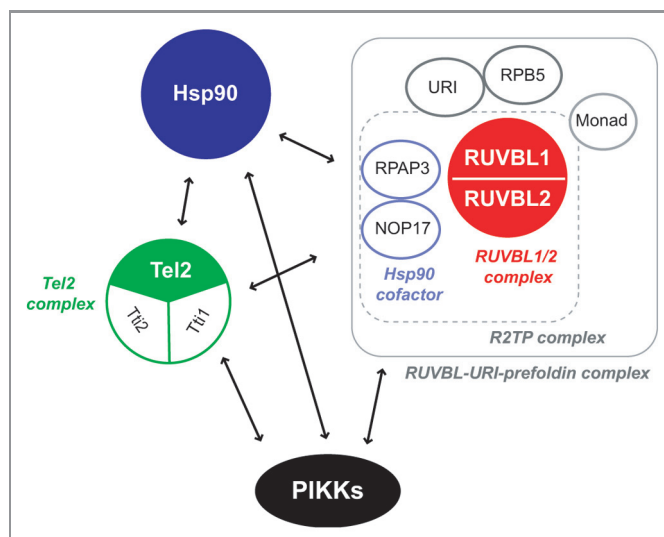

Figure 6. The putative "PIKK regulatory complex." Three common PIKK regulators, the RUVBL1/2 complex, Hsp90 and the Tel2 complex interact with one another. Other factors (RPAP3, NOP17, RPB5, URI and Monad) are shared interactors of the RUVBL1/2 complex, Hsp90 and the Tel2 complex. They are possible PIKK regulators (see **Table 1**). The interaction between the RUVBL1/2-URI-prefoldin complex and the Tel2 complex is mediated by NOP17 in a Tel2 phosphorylation dependent manner.[166]

cancer by inducing tumor immunity.[173] In addition to the regulation of all PIKKs, the RUVBL1/2 complex is implicated in telomerase activity and the Hsp90 pathway,[83,99] both of which are promising targets of cancer therapy and the inhibitors of which are under clinical trials.[174,175] RUVBL1 and RUVBL2 are also involved in c-Myc-mediated cellular transformation and cancer metastasis through the transcriptional regulation with β-catenin and the TIP60 HAT complex.[80,176] Thus, the RUVBL1/2 complex represents a molecular target for cancer therapy through the simultaneous suppression of the above mentioned multiple pathways. In support of this idea, suppression of the RUVBL1/2 complex induces growth arrest and increased apoptosis of tumor cells in vitro and in vivo.[177]

Conclusions and Perspectives

While much is known about the critical importance of PIKKs in cellular stress responses, their overall regulatory mechanisms and the interplay among PIKKs are not well defined. The finding that all PIKKs are regulated by common regulators provides important insights into these issues.

A common PIKK regulator, the RUVBL1/2 complex, can regulate each PIKK function by controlling PIKK levels and through physical interaction with each PIKK. This suggests that the RUVBL1/2 complex mediates PIKK signaling and coordinates each PIKK-mediated stress response as a common PIKK regulator. Based on its diverse cellular functions,[74] the RUVBL1/2 complex possibly links the PIKK-mediated stress responses to other cellular processes, thereby facilitating correct stress responses. Although the molecular mechanisms of the RUVBL1/2

Table 1. List of common and possible PIKK regulators in mammals

	Molecule	Domain/Motif	Character and related cellular process	Possible phosphorylation site by PIKKs**
Common PIKK regulators	RUVBL1 (RuvB-like 1)	AAA+ domain, Walker A, WalkerB motif	AAA+ family proteins, ATPase/DNA helicase activity, form a hexameric complex, transcriptional regulation, RNA modification/biogenesis, telomere maintenance, DNA repair, spindle formation, Hsp90 cofactor, NMD	-
	RUVBL2 (RuvB-like 2)			Prediction & report: Ser220 (ref. 105)
	Hsp90 (Heatshock protein 90)	Histidine kinase-like ATPases domain	conserved molecular chaperone, ATPase, promotes protein folding/structural maturation/assembly/transport of specific client proteins	Prediction & report: Thr297 (ref. 105)
	Tel2 (telomere maintenance 2)	-	replication checkpoint, DNA damage response/checkpoint	-
	SMG-10/Tti1 (Tel2 interacting protein 1)	HEAT repeat	Tel2 complex component, DNA damage response/checkpoint	prediction: Ser391
	Tti2 (Tel2 interacting protein 2)	-	Tel2 complex component, DNA damage response/checkpoint	-
Possible PIKK regulators	RPAP3 (RNAPII-Associated Protein 3)	TPR motif	RNA polymerase associated protein, Hsp90 cofactor, UV-induced DNA damage response and cell survival, TNF-α and cycloheximide-induced apoptosis	prediction: Ser116 Ser481
	NOP17 (Nucleolar protein 17)	PIH1 domain	pre-rRNA processing/RNA modification, Hsp90 cofactor	-
	URI/RMP (Unconventional prefoldine RPB5 interactor/RPB5 mediating protein)	Prefoldin α domain	unconventional prefoldin, transcriptional regulation, regulation of survival signaling at mitocochondria	phosphrylated at Ser371 by p70 S6K, downstream of mTOR (ref. 161)
	RPB5 (RNA polymerase II subunit 5)	-	shared subunits of all three RNA polymerases, transcriptional regulation, NMD	prediction: T29
	Monad/WDR92 (WD repeat domain 92)	WD40 domain	RNA polymerase associated protein, TNF-α and cycloheximide-induced apoptosis	-

Notes: *All molecules are evolutionarily conserved in eukaryote; **possible phosphorylation sites were predicted by the scansite program (http://scansite.mit.edu/) with high stringency. Aabbreviations; AAA+, ATPase associated diverse cellular activities; TPR, tetratricopeptide repeat; HEAT, Huntingtin, elongation factor 3, A subunit of protein phosphatase 2A, and TOR1.

complex-mediated PIKK regulation remain to be fully elucidated, recent studies have indicated the existence of putative PIKK regulatory chaperone complexes, including the RUVBL1/2 complex, the Tel2 complex and Hsp90. Future studies will clarify the PIKK-regulatory roles of the RUVBL1/2 complex as an ATPase and the function of the Tel2 complex in the chaperone activity of Hsp90. The putative PIKK regulatory chaperone complex contains additional factors, and their function and PIKK preference should also be examined. In addition, global analyses to evaluate the interplay among PIKKs and the linkage of PIKK signals to other cellular processes are important. Further analyses will reveal the physiological significance of the common regulators of PIKKs and help our understanding of the basic mechanisms underlying proper stress responses in living organisms.

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