UPF1
A leader at the end of chromosomes

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The human helicase and ATPase upframeshift suppressor 1 (UPF1), traditionally known as a major player in several RNA quality control mechanisms, is emerging as a crucial caretaker of the stability of the genome. Work from my laboratory has provided insight into the function of UPF1 during DNA metabolism and has revealed that this versatile enzyme sustains the proper replication of telomeres, the protective structures located at the ends of linear eukaryotic chromosomes. We have supplied direct evidence that telomere replication is not completed in cells with compromised UPF1 function, leading to the accumulation of DNA damage and telomere abnormalities. We also have isolated a number of factors that physically interact with UPF1 and might represent molecular links between UPF1 and telomeres. In this paper, I re-evaluate the functions of UPF1 in maintaining the stability of telomeres and of the genome at large and suggest a model that explains how UPF1 might be recruited and function during telomere replication.

The functions of UPF1 (also known as suppressor with morphogenetic defects in genitalia 2, SMC2, and regulator of nonsense transcripts 1, RENT1) have been analyzed largely in the context of mRNA (mRNA) quality control mechanisms, mostly taking place in the cytoplasm. As already mentioned, NMD execution occurs mostly in the cytoplasm. Consistently, indirect immunofluorescence (IF) experiments performed with antibodies raised against human UPF1 have revealed that the majority of UPF1 molecules are dispersed diffusely through the cytoplasm of human cancer cells. Nevertheless, a faint but reproducible staining of the nucleus is also observed (for an example, see Fig. 1). The use of leptomycin B to block chromosomal region maintenance 1 (CRM1)-mediated nuclear export leads to the rapid nuclear accumulation of endogenous as well as green fluorescence protein (GFP)-tagged UPF1. Moreover, IF staining of cells treated with mild detergents to extract soluble proteins clearly reveals that a substantial fraction of insoluble UPF1 molecules are dispersed diffusely through the cytoplasm of human cancer cells. Nevertheless, a faint but reproducible staining of the nucleus is also observed (for an example, see Fig. 1). The use of leptomycin B to block chromosomal region maintenance 1 (CRM1)-mediated nuclear export leads to the rapid nuclear accumulation of endogenous as well as green fluorescence protein (GFP)-tagged UPF1. Moreover, IF staining of cells treated with mild detergents to extract soluble proteins clearly reveals that a substantial fraction of insoluble UPF1 molecules are dispersed diffusely through the cytoplasm of human cancer cells. Nevertheless, a faint but reproducible staining of the nucleus is also observed (for an example, see Fig. 1). The use of leptomycin B to block chromosomal region maintenance 1 (CRM1)-mediated nuclear export leads to the rapid nuclear accumulation of endogenous as well as green fluorescence protein (GFP)-tagged UPF1. Moreover, IF staining of cells treated with mild detergents to extract soluble proteins clearly reveals that a substantial fraction of insoluble UPF1 is dispersed diffusely through the cytoplasm of human cancer cells. Nevertheless, a faint but reproducible staining of the nucleus is also observed (for an example, see Fig. 1).
that a fraction of nuclear UPF1 stably associates with chromatin. Interestingly, yeast Upf1p is thought to be localized exclusively in the cytoplasm, indicating that putative nuclear functions associated with UPF1 emerged late during evolution and might represent a unique feature of mammalian UPF1 proteins.

Using protein gel blot analysis of chromatin fractions prepared from unperfused, cell-cycle synchronized and UV-irradiated human cultured cells, we were the first to determine that UPF1 associates with chromatin. We showed that UPF1 gamma-irradiated human cultured cells, turbided, cell-cycle synchronized and chromatin fractions prepared from unperfused cells, or pre-treated with mild detergents, to detect insoluble UPF1 (lower panels). UPF1 antibodies were revealed using secondary antibodies conjugated with Alexa 488 fluorochrome (shown in green), whereas DNA was stained using DAPI (in blue). Note the focal staining of insoluble UPF1 in the nucleus. Scale bars correspond to 10 μm.

Our studies have set the stage for the detailed characterization of the role of UPF1 in maintaining the stability of telomeres. Still, a number of crucial questions remain to be addressed experimentally to fully understand the details of the telomeric functions linked to UPF1. It is unclear, for example, exactly how UPF1 helps to maintain genome stability came from experiments employing short hairpin RNA (shRNA) plasmids to deplete UPF1 in human cancer cells. UPF1 depletion leads to the accumulation of nuclear DNA repair foci, which contain the DNA damage marker γH2AX and the single-stranded DNA binding protein replication protein A (RPA). Importantly, the depletion of UPF2, another NMD effector, does not trigger a DNA damage response although it impairs NMD execution to levels similar to that of UPF1. This suggests that inefficient canonical NMD does not compromise genome integrity and reveals alternative roles for UPF1 in preserving genome integrity that do not depend on NMD. The DNA damage response evoked by UPF1 depletion was followed by cell-cycle arrest at the onset of the S-phase, accompanied by the accumulation of proliferating-cell nuclear antigen (PCNA) molecules in the cell nucleus, in a distribution pattern that is typical of the early S-phase. In addition, the γH2AX accumulation induced by UPF1 depletion was prevented when cells were concomitantly depleted of the ataxia-telangiectasia and Rad3-related (ATR) protein, a phosphoinositide-3-kinase-related protein kinase (PIKK) that is a major player in signaling and stabilizing arrested replication forks. In successive studies, we have shown that UPF1-depleted cells accumulated telomeric aberrations, including telomere-free chromosome ends and fragile, shredded telomeres, preferentially at chromatids replicated by the leading-strand semi-conservative replication machinery. Based on this experimental evidence, we were the first to propose that UPF1 is required for the complete replication of telomeric DNA (see model in Fig. 2) and that, strikingly, UPF1 association with chromosomal DNA is replicated, and upon repair, UPF1 co-localizes with telomeric heterochromatin.

Recent studies have revealed that UPF1 also associates with actively transcribing, PTC+-reporter genes that have been stably integrated into the genome of human cancer cells. The association of UPF1 with transcribed PTC-containing genes is thought to prevent the splicing and export of PTC-containing pre-mRNAs. On the other side, the in vivo association of UPF1 with telomeres constituted the primary concrete and direct evidence that UPF1 is involved in regulating telomeric DNA metabolism.

The first indication that UPF1 helps to maintain genome stability came from experiments employing short hairpin RNA (shRNA) plasmids to deplete UPF1 in human cancer cells. UPF1 depletion leads to the accumulation of nuclear DNA repair foci, which contain the DNA damage marker γH2AX and the single-stranded DNA binding protein replication protein A (RPA). Importantly, the depletion of UPF2, another NMD effector, does not trigger a DNA damage response although it impairs NMD execution to levels similar to that of UPF1. This suggests that inefficient canonical NMD does not compromise genome integrity and reveals alternative roles for UPF1 in preserving genome integrity that do not depend on NMD. The DNA damage response evoked by UPF1 depletion was followed by cell-cycle arrest at the onset of the S-phase, accompanied by the accumulation of proliferating-cell nuclear antigen (PCNA) molecules in the cell nucleus, in a distribution pattern that is typical of the early S-phase. In addition, the γH2AX accumulation induced by UPF1 depletion was prevented when cells were concomitantly depleted of the ataxia-telangiectasia and Rad3-related (ATR) protein, a phosphoinositide-3-kinase-related protein kinase (PIKK) that is a major player in signaling and stabilizing arrested replication forks. In successive studies, we have shown that UPF1-depleted cells accumulated telomeric aberrations, including telomere-free chromosome ends and fragile, shredded telomeres, preferentially at chromatids replicated by the leading-strand semi-conservative replication machinery. Based on this experimental evidence, we were the first to propose that UPF1 is required for the complete replication of telomeric DNA (see model in Fig. 2) and that, strikingly, UPF1 association with chromosomal DNA is replicated, and upon repair, UPF1 co-localizes with telomeric heterochromatin. Recent studies have revealed that UPF1 also associates with actively transcribing, PTC+-reporter genes that have been stably integrated into the genome of human cancer cells. The association of UPF1 with transcribed PTC-containing...
performing as a canonical replicative helicase. This hypothesis is supported by the fact that UPF1 physically interacts with independent subunits of the replicative DNA polymerase delta (polδ, Fig. 2).16,28 In apparent contrast with this hypothesis is the fact that the telomeric dysfunctions observed in UPF1-depleted cells mostly derive from defects in leading-strand semi-conservative replication,25 whereas polδ is known to act within the lagging-strand replication machinery. Nevertheless, it remains to be determined if UPF1 also associates to the leading-strand replication machinery, perhaps through interaction with DNA polymerase epsilon (polε; Fig. 2). Indeed, UPF1 might be required for both leading- and lagging-strand replication of telomeric DNA, as suggested by the slight but consistent accumulation of fragility also at lagging-strand telomeres.25 The more prevalent accumulation of leading-strand fragile telomeres could indicate that UPF1 action is more important for leading-strand replication or that defects in telomere lagging-strand replication occur with equal frequency but are more efficiently buffered and repaired, perhaps by telomerase, a specialized reverse transcriptase that is able to synthesize telomeric repeats de novo and to add them to the 3’ end of telomeres.25,29 It will be interesting to re-analyze the strand specificity of the telomeric defects that arise upon UPF1 depletion in cells in which telomerase activity has been inhibited.

Given the well-established connection between UPF1 and RNA metabolism, UPF1 might sustain telomere replication by acting on telomeric repeat-containing non-coding RNA (TERRA), a recently discovered type of RNA (TERRA), a recently discovered type of RNA. TERRA molecules are transcribed in a centromere-to-telomere direction by DNA-dependent RNA polymerase II (RNAPII), which uses the C-rich telomeric strand as a template. TERRA molecules, therefore, contain G-rich telomeric RNA repeats. RNA polymerase II (RNAPII), which uses the C-rich telomeric strand as a template. TERRA molecules, therefore, contain G-rich telomeric RNA repeats. A large fraction of human TERRA is transcribed from CpG dinucleotide-rich promoters located in subtelomeric regions 250–1,000 bp away from the first telomeric repeat, and remains post-transcriptionally associated with telomeres through mechanisms that could involve telomeric repeat binding factors 1 and 2 (TRF1 and TRF2, respectively), which are components of the multiprotein telomeric complex shelterin (Fig. 2), and telomerase.25,26,28,29 Indeed, TERRA was shown to interact with these three factors in cell extracts and in vitro.25,26 The constant presence of an RNA moiety consisting of telomeric RNA repeats could halt the progression of the replication fork through the telomere by associating with
the single-stranded telomeric DNA that is transiently generated during replication, either by directly base pairing with the C-rich telomeric strand or by forming G-quadruplex intermediates with the G-rich telomeric strand.36,37 The helicase activity of UPF1 might therefore displace TERRA from telomeric ribonucleoprotein complexes -possibly containing TRF1, TRF2 and telomerase- or directly resolve RNA–DNA hybrids that form transiently at the telomeric replication fork.37

Supporting a model in which UPF1 physically displaces TERRA from telomeres (Fig. 2), we have shown that cells mildly depleted for UPF1 by stable infection with shRNA-expressing lentivirus accumulate TERRA molecules at telomeric heterochromatin in the absence of detectable changes in the half-life of TERRA.14-16 However, the accumulation of TERRA may be a consequence rather than a cause of the telomere dysfunctions induced by UPF1. Telomere uncapping induced by TRF2 knockdown or exposure to telomere G-strand DNA oligonucleotides has been shown to increase TERRA steady-state levels through pathways requiring the tumor suppressor protein p53.36 Therefore, TERRA accumulation might be part of the complex DNA damage response evoked by uncapped telomeres that may occur in UPF1-deficient cells.

Another intriguing question raised by observations in my laboratory concerns the functional significance of the physical interaction between UPF1 and telomerase (Fig. 2). Both endogenous and ectopically expressed epitope-tagged UPF1 molecules physically interact with telomerase in human cancer cell nuclear extracts, independently of nucleic acid molecules.39 It is unclear if this interaction is directly established between UPF1 and human telomerase reverse transcriptase (hTERT), the catalytic subunit of the telomerase holoenzyme.40 Like UPF1, the human ever-smaller telomere protein A (EST1A, also known as SMG6)—an NMD effector that interacts directly with UPF1—directly associates with telomerase through protein–protein and protein–RNA interactions with hTERT and human telomerase RNA (hTR), the RNA moiety of the telomerase holoenzyme, respectively (Fig. 2).41-43 EST1A, therefore, may mediate UPF1/ hTERT interaction; in vitro experiments using recombinant proteins and immunoprecipitation experiments performed in cells depleted for either factor will help clarify this issue. In addition, we also have shown that depletion of ATR strongly reduces the amount of active telomerase co-immunoprecipitating with UPF1 without affecting the total cellular activity of telomerase.44 Therefore, ATR may mediate UPF1/ telomerase interaction either by creating a physical bridge between the two molecules or, more likely, by modifying the UPF1 phosphorylation state (see below). Regardless of how the interaction between UPF1 and telomerase is established, a hint suggesting its functional relevance comes from the observation that cells overexpressing an hTERT allele C-terminally fused to a human influenza hemagglutinin tag (hTERT-HA) fail to properly recruit UPF1 to telomeres.37 Although it is catalytically active in vitro, hTERT-HA is unable to elongate telomeres in vivo, possibly because tag interference prevents the proper recruitment of the protein to telomeric loci.45 Therefore, overexpressed nucleoplasmic hTERT may tether UPF1 away from telomeric loci, suggesting that telomerase may be involved in recruiting UPF1 at telomeres.

The interaction between UPF1 and telomerase may also constitute a regulatory step in telomerase activity. The Saccharomyces cerevisiae helicase petite integration frequency 1 (Pif1p) interacts with telomerase and negatively regulates telomerase-mediated telomere elongation and telomere healing of intrachromosomal DSBs.46-48 Pif1p is thought to negatively regulate telomerase-mediated elongation of telomeres by unwinding the template region of telomerase component 1 (TLC1) RNA from single stranded telomeric 3’ ends.49 Remarkably, although the mutine homologue of Pif1p interacts with telomerase at low levels, it does not play a major role in either regulating telomerase-mediated telomere elongation or telomere healing.50 Another helicase, perhaps UPF1, might have acquired the yeast Pif1p telomeric functions in mammalian cells. The generation of UPF1 alleles mildly depleted for UPF1 by stable infection with gamma rays or left untreated.51 Human UPF1 is a phospho- protein containing 28 consensus sites for PIKK-mediated phosphorylation (Serine/Threonine-Glutamine), 14 of which are clustered within its C-terminal amino acids. ATR is able to directly phosphorylate UPF1 in vitro, although the specific substrate residues on UPF1 still need to be identified, and ATR depletion leads to a substantial decrease in cellular phosphorylated UPF1.52 Therefore, it is likely that ATR-mediated phosphorylation of UPF1 is the molecular event contributing to UPF1 loading or stabilization at telomeres (Fig. 2). Indeed, ATR was shown to localize to telomeric heterochromatin in human cells transiting through the S-phase,53 suggesting that, at telomeres, ATR could act locally on UPF1 to maintain or activate it. It will be interesting to identify the phosphorylation residues of UPF1 that are modified by ATR and test whether abrogation of such phosphorylation indeed prevents the localization of UPF1 at telomeres and onto the chromatin of replicating cells.

We have now reported a novel physical interaction between UPF1 and the shelterin component TPP1 (derived from the three acronyms TIN1, POTT, and PIP1)19,20,25. TPP1 is an integral component of the telomeric heterochromatin and is recruited at telomeres through direct
interaction with TRF1-interacting protein 2 (TRF2), another shelterin factor. TRF2, in turn, binds to telomeres through interaction with TRF1 and TRF2 (Fig. 2). UPF1 interaction with TPPI, which was demonstrated both using endogenous and ectopically expressed proteins, depends on the presence of an N-terminal oligonucleotide/oligosaccharide binding (OB) domain in TPPI. 18 TPPI-mediated depletion of TPPI did not substantially alter the telomeric localization of UPF1, suggesting that TPPI is not a recruitment factor for UPF1 (our unpublished observations); however, the remaining cellular levels of TPPI after siRNA treatment might be sufficiently high to promote UPF1 binding to telomeric chromatin. The use of TPPI gene knockout systems will help clarify this particular issue. Still, TPPI may regulate UPF1 activity at telomeres during replication rather than recruiting or stabilizing it. Interestingly, TPPI and the protection of telomeres 1 (POT1) protein form a complex that directly associates with active telomerase and regulates telomerase activity at telomeres. 19 An appealing hypothesis is that UPF1 may match the same complex and contributes to the TPPI/POT1-mediated regulation of telomerase.

A final, more general question is whether UPF1 is involved in the replication of genomic loci other than telomeres. The foci detected in the nucleus of pre-extracted cells in UPF1 IF experiments are unlikely to correspond to telomeres alone (Fig. 1). In addition, and as noted previously, UPF1 depletion leads to the accumulation of nuclear foci containing hYHAX and RPA, only a fraction of which co-localize with telomeric markers. 19 These observations indicate that UPF1 does not accumulate only at telomeres but instead broadly associates with different chromatin domains and promotes the stability of different genomic loci. The large-scale identification of the genomic regions that are preferentially bound by UPF1 and of those that become unstable upon UPF1 depletion is necessary to unravel the general function of UPF1 on chromatin. It is conceivable that UPF1 could promote the replication of a number of genomic regions sharing structural features with telomeres. For example, UPF1 might be generally required for the replication of repetitive DNA sequences or of heterochromatic loci constitutively associated with RNA moieties. Careful characterization of the common features of the genomic loci requiring UPF1 for their replication will completely reveal the nuclear functions of this versatile helicase.

It is now accepted that UPF1 has multiple cellular roles that allow it to promote both RNA and DNA quality control. The intimate connection of UPF1 with genomic stability executioners such as ATR and the severe DNA damage occurring at telomeric and related to telomeres, in UPF1-depleted cells place the protein among the cellular caretakers of genomic integrity. The double duties of UPF1 in RNA and DNA metabolism suggest a scenario in which UPF1 coordinates the stability of nucleic acids both in the nucleus and in the cytoplasm. A careful dissection of the functional relevance of the interactions between UPF1 and its different interaction partners, including ATR, TPPI, and telomerase, as well as the development of functionally separate UPF1 alleles will help appreciate the multifaceted functions of this versatile enzyme.

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