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Xeroderma Pigmentosa Group A (XPA), Nucleotide Excision Repair and Regulation by ATR in Response to Ultraviolet Irradiation

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Abstract

The sensitivity of *Xeroderma pigmentosa* (XP) patients to sunlight has spurred the discovery and genetic and biochemical analysis of the eight XP gene products (XPA-XPG plus XPV) responsible for this condition. These studies also have served to elucidate the nucleotide excision repair (NER) process, especially the critical role played by the XPA protein. More recent studies have shown that NER also involves numerous other proteins normally employed in DNA metabolism and cell cycle regulation. Central among these is ataxia telangiectasia and Rad3-related (ATR), a protein kinase involved in intracellular signaling in response to DNA damage, especially replicative and transcription stresses. This review summarizes recent findings on the interplay between ATR as a DNA damage signaling kinase and as a novel ligand for intrinsic cell death proteins to delay damage-induced apoptosis, and on ATR's regulation of XPA and the NER process for repair of UV-induced DNA adducts. ATR's regulatory role in the cytosolic-to-nuclear translocation of XPA will be discussed. In addition, recent findings elucidating a non-NER role for XPA in DNA metabolism and genome stabilization at ds-ssDNA junctions, as exemplified in prematurely aging progeroid cells, also will be reviewed.

Introduction

Individuals with mutations in *Xeroderma pigmentosa* (XP) genes are especially sensitive to the ultraviolet (UV) rays (180–315 nm) in sunlight [1, 2]. These individuals accumulate DNA damage in their skin cells after solar irradiation, primarily as a cyclobutane pyrimidine dimer (CPD) and, to a lesser extent, as a (6–4) photoproduct ((6–4) PP). Normally, these intrastrand cross-links of adjacent pyrimidine bases are removed from the DNA by nucleotide excision repair (NER) [1, 3–5]. In addition to other repair factors, seven XP gene products are involved in the NER process: *Xeroderma pigmentosa* complementation groups A through G (XPA – XPG). Mutations in any of these XP gene products reduces the efficiency of this repair process with XPA and XPC mutations being the most frequent [6] and XPA deficiency showing the highest sensitivity to UV [7]. If adducts persist they may be

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bypassed by error-prone translesion synthesis using DNA polymerase eta (Pol η), a product of the XPV (polH) gene [8]. The structure and mutational features, plus post-translational modifications of these XP proteins have been reviewed recently by Feltes and Bonatto [9]. XPA mutation is the most severe XP deficiency is since this protein is required in both the global genomic NER (GG-NER) and the transcription- coupled NER (TC-NER) sub-pathways of nucleotide excision repair [10–1415]. XPC mutations, though relatively frequent, are less severe since this protein is primarily involved in GG-NER [10,16]. Though not an XP protein, the DNA damage checkpoint protein ataxia telangiectasia and Rad3-related (ATR) also is essential for initiation and regulation of the NER process [17, 18]. Thus, this review will focus on new information from the last decade on the biochemical roles and cellular mechanisms of XPA and ATR in the nucleotide excision repair process and cell death, and discuss recent findings on possible non-NER functions of XPA in both the nucleus and in the cytoplasm.

ATR signaling mediates the cellular response to DNA damaged induced by ultraviolet radiation

The presence of UV-induced CPD and (6–4) PP adducts in mammalian nuclear DNA generates a cascade of events as part of the DNA damage response (DDR). Generally, these helix-distorting, replication- and transcription-blocking DNA adducts induce activation of the DNA repair process and arrest the cell cycle to allow for repair of the damaged DNA. ATR, a key regulator of these processes, is a member of the phosphatidylinositol 3-kinase (PI3K) family. The PI3K family of protein kinases also includes the other stress-responsive protein kinases *ataxia telangiectasia* mutated (ATM), DNA-dependent protein kinase (DNA-PK) and mammalian target of rapamycin (mTOR) [19, 20]. Although it functions in multiple DDR processes [21] ATR is the primary regulator of the nucleotide excision repair pathway due to its ability to detect the replicative and transcriptional stresses caused by UV-induced damage and other bulky DNA adducts resulting from chemical toxins and some chemotherapeutic agents [22–25].

Induction of CPDs and (6–4) PPs in DNA generates obstacles to DNA replication and transcription. The resulting replicative and transcriptional stresses stall DNA polymerization during replication and pol II progression in RNA synthesis[11, 12], respectively, leading to an accumulation of stretches of single-strand DNA (ssDNA), which become coated with the ssDNA-binding replication protein A (RPA) [26]. ATR in complex with its nuclear binding partner ATR-interacting protein (ATRIP) binds to this RPA-coated ssDNA via an ATRIP-RPA interaction. ATRIP also serves to activate the checkpoint kinase activity of ATR [4, 27–30]. Activated ATR kinase phosphorylates many downstream mediators/effectors which include checkpoint kinase 1 (Chk1), A-kinase-anchoring protein 12 (AKAP12), p38/mitogen-activated protein kinase-activated protein (MAPKAP) kinase 2 (MK2), the tumor suppressor protein p53, ATRIP and XPA [27, 31–33]. Phosphorylation activates these downstream proteins resulting in arrest of cell cycle progression, activation of DNA repair and, in cases of severe damage, apoptotic cell death [22, 34, 35]. ATR is an essential gene for the initiation and regulation of NER and for genome maintenance [17, 36, 37].

Historically, ATR has been described as a necessary protein kinase which functions in the cell nucleus to regulate DNA replication and various responses to DNA damage and cellular stress [38, 39]. Possible non-nuclear roles for ATR have received little attention. However, a recent study described an anti-apoptotic, cytoplasmic role for ATR [40, 41]. It was demonstrated that in mammals a small fraction of cellular ATR normally exists in the cytoplasm (cytoATR) and that, in response to DNA damaging agents, the amount of this cytoATR increases and changes conformation, resulting in a slower-migrating, higher electrophoretic band (ATR-H) as compared with the faster-migrating, lower electrophoretic band (ATR-L). The most efficient induction of ATR-H formation was by UV irradiation, though it also was induced by camptothecin and hydroxyurea, agents which cause DNA double-strand breaks (DSBs). Interestingly, the increase in cytoATR appears to result from nuclear export and not from new protein biosynthesis [41]. This nuclear export of ATR-L and its conversion to cytoplasmic ATR-H by UV irradiation was observed in normal human fibroblasts, transformed skin keratinocytes, multiple human cancer cell lines, and in transformed mouse embryonic fibroblasts [40].

It was found that the ATR-L is a prolyl *trans*-isomer of cytoplasmic ATR while ATR-H is the *cis*-isomer [40]. The formation of cytoplasmic ATR-L (*trans*-ATR) from ATR-H (*cis*-ATR) is mediated by peptidylprolyl cis/trans isomerase NIMA-interacting 1 (Pin1) [40]; this enzyme is a critical regulator of many biological processes in both normal and diseased cells [42–48]. Since ATR is naturally more stable in its *cis*-isomeric form, newly-synthesized ATR is in the ATR-H isoform but is quickly converted to the ATR-L isoform by Pin1 isomerization of the phospho- Ser^{428}--Pro^{429} site of the ATR protein [40]. This isomerization converts Pro^{429} from the *cis*- (ATR-H) to the *trans*-isoform (ATR-L). Surprisingly, this conformational change of only one out of 2,644 amino acids is sufficient to reduce the electrophoretic mobility of the ATR protein in 3–8% gradient SDS-polyacrylamide gels, similar to adding ~10 kilodaltons, to generate a clearly distinguishable higher band (ATR-H). The mechanism of this protective response stems from UV-induced changes in the phosphorylation status of the $Ser^{428}Pro^{429}$ site in ATR and the Ser^{71} residue in Pin1. UV irradiation induces DAPK1 to phosphorylate Pin1 at Ser^{71}, thus inactivating the isomerase activity [49, 50]. The UV irradiation also induces a dephosphorylation of the phospho-Ser^{428}-Pro^{429} site in ATR, rendering it a non-recognizable Pin1 site [40]. Together, these changes in phosphorylation status allow cytoATR to assume the *cis* isoform, ATR-H. Although the details of the UV-induced changes in DAPK1 kinase and the unknown phosphatase activities remain to be elucidated these observations reveal a very sensitive cellular sensor for ultraviolet damage and ATR isomeric conversion.

Upon UV irradiation-induced DNA damage ATR initiates the nuclear NER process to repair the genome. To allow time for completion of this repair the cell needs to stall two processes: cell cycle progression, especially through S phase, and the initiation of damage- induced cell death. Cell cycle arrest is needed to allow time for DNA repair and, thus, prevent the introduction of mutations by replication through unrepaired CPD and (6–4) PP damage sites. A classic feature of ATR in response to UV damage is its phosphorylation of Chk1 kinase, which then phosphorylates other proteins to arrest cell cycle progression [51]. UV-induced damage also can activate the intrinsic cell death pathway through the release of mitochondrial cytochrome C into the cytosol which activates caspase cleavage and eventual

apoptosis [52]. But how does ATR stall the onset of apoptotic cell death to allow sufficient time for cell recovery by repair of the CPD and (6–4) PP damage? The answer lies in the interaction of cytosolic ATR-H with the proapoptotic protein tBid (truncated BH3 interacting-domain death agonist) as described by Hilton *et al.* [40]. In response to damage Bid promotes polymerization of proapoptotic proteins Bax (bcl-2-associated X) and Bak (bcl-2 homologous antagonist-killer) at the mitochondrial surface, which induces cytochrome C release leading to apoptotic cell death [52]. Hilton *et al.* surprisingly found that ATR contains a BH3-like domain which allows it to function like a prosurvival Bcl-2 family protein. In the nucleus, ATR remains in the form of ATR-L, regardless of UV, whose BH3 domain appears to be masked in a folded N-terminal region of the *trans*- isoform protein; however, the N-terminus is unfolded in the cytosolic *cis*-isoform which exposes this BH3 domain, allowing ATR-H to bind to and sequester tBid protein, thus delaying initiation of the intrinsic cell death pathway [40]. Figure 1 illustrates how the *cis*- and *trans*-isoforms may affect these changes in the accessibility of the BH3 domain in ATR-L vs. ATR-H isoforms, and how the ATR-L form is necessary for the regulation of XPA import and NER efficiency.

Nuclear ATR is well known for its association with ATRIP, a necessary interaction which activates the kinase activity of ATR in addition to localizing it to the RPA-coated ssDNA at damage sites [4, 27–30]. This kinase activity is essential for ATR's activation of downstream proteins during the DDR. In contrast, Hilton *et al.* found that cytoATR is free of ATRIP, which remains sequestered in the nucleus after UV irradiation. Also, the anti-apoptotic function of mitochondrial ATR-H is independent of its checkpoint kinase activity [40, 41]. Thus, the regulated *cis*- *vs. trans*-isoform switching between ATR-H and ATR-L allows distinct prosurvival functions of ATR in the cytoplasm versus those in the nucleus in response to UV irradiation. Particularly, the cytoplasmic ATR-H prevents premature cell death at mitochondria. This coordination of the cytoplasmic antiapoptotic and the nuclear cell cycle arrest/DNA repair roles provides time for damage repair before any decision on programmed cell death needs to be made. Note that, once formed, ATR-H reaches a maximum within 2 hours but persists in the cytoplasm for over 8 hours, sufficient time for most NER-competent cells to repair all the (6–4) PP adducts and most, if not all, of the CPD adducts [40, 53,41]. Thus, this slow re-isomerization of ATR-H to ATR-L may serve as an internal timer of repair efficiency and death.

The novel finding of the cytoplasmic role of ATR as an anti-apoptotic protein at mitochondria highlights that much remains to be discovered about the signaling molecules involved in the DNA damage responses. These observations support previous findings that prolyl isomerization of a single residue in a large protein may have pleotropic effects on a protein's structure and function [48, 54]. Also, these cytoplasmic prosurvival functions are not only novel for ATR since ATM also displays similar stress functions at peroxisomes in response to increased levels of reactive oxygen species [55–59] and at mitochondria in response to DNA damage [60, 61].

ATR-XPA interactions are necessary for the nuclear import of XPA and for efficient nucleotide excision repair

The data sheets accompanying nearly all commercial anti-XPA antibodies recommended for immunofluorescence studies by the suppliers indicate that XPA is a protein located in the nucleus only. This discrepancy stems from the early studies in which formalin (2– 4% *para*-formaldehyde) was used for cell fixation [62, 63]. More recent immunofluorescence studies of XPA's subcellular distribution confirmed that in *para*-formaldehyde-fixed cells the endogenous protein was observed to be nuclear [64–66]. However, biochemical fractionation of millions of cells into nuclear vs. cytoplasmic fractions revealed that XPA occurs predominantly in the cytoplasm of normal mammalian cells and that it is translocated to the nucleus in response to DNA damage, especially from UV irradiation [67–70]. These biochemical findings were confirmed by immunofluorescence observations of methanol-fixed cells [67–70]. We have observed that with either fixative the anti-XPA antibodies revealed XPA in the nucleus, but antibody detection of the cytosolic XPA occurred only in cells fixed with cold methanol. Methanol fixation extracts lipids, dehydrates and permeablizes cells causing proteins to denature and precipitate onto the cellular architecture. In contrast, *para*-formaldehyde fixation cross-links proteins and other macromolecules in place [71]. A possible explanation, then, for the reported differences in the subcellular localization of XPA with these two methods is that methanol fixation disrupts the cloaking interaction between XPA and an as yet undescribed cytosolic XPA binding protein (cXBP) which sequesters XPA in the cytoplasm; the methanol fixation with denaturation then exposes XPA's antigenic site; in contrast, *para*-formaldehyde fixation locks this XPA-cXBP complex in place, thus masking the XPA epitopes in the cytosol. UV irradiation induces a disruption of this cytosolic XPA-cXBP complex, releasing XPA for nuclear import and detection in nuclei of cells fixed with methanol or *para*-formaldehyde. This also could be true for other so- called nuclear proteins.

Wu *et al.* reported that ATR regulated XPA nuclear import in response to UV radiation [67,72]. More recent studies by Li *et al.* have revealed further important details of the cytosol-nuclear translocation of XPA. The tumor suppressor protein p53 is a major downstream effector molecule and phosphorylation substrate in the ATR-mediated DDR. In support of earlier observations [5367], Li *et al.* demonstrated that the nuclear import of XPA in response to UV irradiation or cisplatin treatment is ATR-dependent in normal fibroblasts and in cancer cells that are p53 proficient; XPA import also is dependent on the transcriptional activity of p53 in these cells [69, 70]. In addition, this dependence on ATR checkpoint activity is cell-cycle phase dependent, occurring only during the S phase [69]. Most XPA remained sequestered in the cytosol in the G_1 phase even after UV treatment; in contrast, in G2-phase cells the nucleus contained the majority of the XPA molecules irrespective of UV irradiation. Consistently, NER recently was found to recruit ATR to the UV-damage sites and to activate ATR in G1-phase but not in S-phase [73–76]. Regulation of S-phase cytosolic XPA translocation into the nucleus by ATR is consistent with previous findings that the peak activity of this checkpoint kinase occurs in S phase as part of normal DNA replication and also in response to DNA damage [34, 77]. Li *et al.* observed that the maximum UV-induced phosphorylation of Ser^{15} of p53 occurred in S phase and that the NER removal of CPD adducts also was most efficient in S phase [69]. Recall that ATR binds to XPA *via* the Lys^{188} and Ser^{196} residues in its HTH

motif containing [53] and that these residues are important for the efficient repair of CPD adducts.

Interestingly, the p53 status of cells significantly influences the role of ATR in regulating DNA repair after UV or cisplatin damage. Although efficient NER removal of the damage was dependent on ATR kinase activity in p53-proficient ($p53^{+/+}$) cells the repair process seemed to be ATR-independent in p53-deficient ($p53^{-/-}$) cells [69, 70, 77]. Consistently, nuclear import of cytosolic XPA is dependent on p53 transcriptional activity in $p53^{+/+}$ cells and occurs much slower in $p53^{-/-}$ cells, but the import stills occurs [70]. Thus, damage-induced ATR activation of the p53 tumor suppressor protein appears to be a primary but not the sole mediator of XPA nuclear import in $p53^{+/+}$ *vs.* $p53^{-/-}$ cells in S phase. The cell cycle checkpoint kinases ATM, Chk1 and MK2 appear not to have a role in XPA nuclear import in $p53^{+/+}$ nor $p53^{-/-}$ cells [69, 70].

The phosphorylation of XPA by ATR is the essential for the NER function of XPA [72]. Shell *et al.* found that ATR binds XPA *via* a specific helix-turn-helix motif in the minimal DNA-binding domain (DBD) and that this XPA motif contains an ATR phosphorylation site (Ser^{196}) [53]. In addition, disruption of this phosphorylation site in XPA with a Ser^{196}Ala mutation significantly reduced the repair efficiency of CPDs but not the repair of (6–4) PPs. The nucleotide excision repair of (6–4) PPs is generally much more efficient than the repair of CPDs [78, 79] and the above finding indicates that ATR's phosphorylation of Ser^{196} in XPA is mechanistically important in the repair of the more prevalent CPDs which represent persistent UV damage. The phosphorylation of Ser^{196} in XPA by ATR appears to stabilize XPA against HERC2-mediated ubiquitinylation and degradation [80].

Shell's structure-function studies also found that the Lys^{188} residue, which is nearby in the same helical DBD of XPA, was critical since a Lys^{188}Ala mutation disrupted the ATR-XPA interaction, thus significantly reducing DNA repair efficiency [81]. Moreover, the normal UV-induced nuclear translocation of cytosolic XPA was lost with the Lys^{188}Ala mutation. However, the Ser^{196}Ala mutation had no effect on XPA's nuclear translocation. The targeting of XPA to the nucleus occurs *via* its nuclear localization sequence (NLS) which contains basic residues located at positons 30–34 of the 273 amino acid protein [62, 63, 82, 68]. This raises the interesting and important question of how XPA is normally held in the cytoplasm if it contains a NLS sequence and its normal NER function is in the nucleus. One possibility is that XPA is sequestered in the cytosol in normal cells *via* association with cXBP, from which it is released for nuclear import after a DNA damaging event such as UV irradiation. Perhaps the stability of the XPA-cXBP complex is disrupted by the phosphorylation of XPA at Ser^{196} and/or by a post-translational modification of the necessary Lys^{188} (i.e., acetylation). Note that highly over-expressed XPA mutants lacking the NLS site can be detected in the cytoplasm by immunofluorescence microscopy in *para*-formaldehyde fixed cells [63], indicating that cXBP may occur is physiologically limiting amounts . This as yet uninvestigated cytosolic XPA sequestration and release could be one of the dynamic components of the UV-induced damage response. Also, note that AKAP12 is normally a cytosolic protein associated with protein kinase A (PKA) but becomes phosphorylated by cytosolic ATR after UV irradiation and then is transported into the nucleus in association with ATR [31].

It is obvious that the DNA damage-induced import of cytosolic XPA into the nucleus is a highly regulated process. Mechanistic features of this import process have been resolved in additional studies by Li *et al.* [68]. It was shown that the NLS in the N-terminal region of XPA was required for nuclear localization. In addition, siRNA knockdown revealed that nucleo-cytoplasmic transport proteins importin-α4 and -α7 were required for XPA nuclear import, but not the other importin-α proteins. Co-immunoprecipitation studies demonstrated that importin-α4 and importin-α7 mediate this nuclear import by direct physical interactions with XPA. However, these two carrier proteins appear to serve different functions during the cell cycle. Importin-α4 transport of XPA was activated by UV radiation and required functional ATR kinase activity, consistent with importin-α4 being responsible for the nuclear import of XPA during the S-phase DNA damage response. In contrast, importin-α7 functioned independent of DNA damage and ATR kinase activation, perhaps reflecting the observed nuclear import of XPA in the G2 phase irrespective of UV exposure [69]. These features of XPA cytosolic localization and cell cycle- dependent nuclear import in response to UV irradiation are diagrammatically summarized in Figure 2.

Nuclear import of proteins requires a GTPase to coordinate protein-protein interactions [83–85]. XAB1 was observed in a yeast two-hybrid system to be an XPA-binding protein with GTPase activity [86]. However, Li *et al.* demonstrated that XAB1 is not the GTPase involved in XPA nuclear import [68]. Also, questions remain on how XPA is released from cXBP in the cytosolic sequestration complex to bind to the importin-α4 in S phase cells exposed to UV. These authors demonstrated that there was an increase in the XPA available for importin-α4 binding within 30 minutes after UV exposure; however, the mechanistic details of the cytosolic DDR remain to be resolved. In addition, how the cytosolic XPA sequestered by cXBP during G1 and S phases is released in non-irradiated cells for importin-α7-mediated nuclear import in the G2 phase also remains to be elucidated.

Does XPA have a cytosolic function outside of nucleotide excision repair?

Why is the XPA protein localized in the cytosol of normal (non-DNA damaged) cells during G1 and S phases of the cell cycle, but not in the G_2 phase? Does its complex with cXBP provide a cytosolic function in G_1 and S phases, and/or is it sequestered there to prevent interference with ongoing nuclear processes?

In addition to high dermatological sensitivity to sunlight XP patients, especially those with an XPA deficiency, often suffer from neurological deficiencies and an early-aging phenotype [2], likely due to non-NER mechanisms as exogenous, genotoxin-induced bulky adducts would not be a concern. XPA interacts with a variety of XP and other proteins during the DNA repair process in the nucleus [68–70, 87, 8882, 89], but interactions with cytosolic proteins have not been described. Are these non-NER features of XPA deficiency related to XPA's cytosolic location, especially in the G_0/G_1 phase status typical of neurons, cardiomyocytes or other differentiated cell types? Other than the descriptions of its UV-induced cytoplasmic-to-nuclear translocation [53, 67–70], possible XPA binding partners and/or functional roles in the cytosol have received little to no attention. One possibility might be that cXBP, the proposed cytoplasmic sequestration factor to which XPA is bound in normal G_1 and S phase cells, influences abnormal, dis-regulatory activity in $XPA^{-/-}$ cells leading to deleterious

metabolic events. Using a bioinformatics analysis Fang *et al.* observed that the XPA$^{-/-}$ phenotype includes neurological features similar to mitochondrial diseases, and results in abnormal mitochondrial energy metabolism, even though cytoplasmic XPA in XPA-proficient cells was absent from the mitochondrial matrix [90]. They also reported increased poly(ADP-ribose) polymerase 1 (PARP1) activity, resulting in higher parsylation of cellular proteins resulting in NAD+ depletion, thus reducing mitochondrial energy generation. They observed that the reduced level of NAD+ downregulated SIRT1, a NAD^+- dependent deacetylase involved in regulating mitochondrial homeostasis and XPA repair activity [91–93]. Fang *et al.* assumed that the PARP1 was activated in XPA$^{-/-}$ cancer cells and neurons by an increased level of basal nuclear DNA damage [90]. However, the presence of a basal level and the type of DNA damage occurring in the XPA–/– cells was not demonstrated. In addition, as reviewed by Weaver and Yang [94], PARP1 activation can be induced by stress responses other than DNA damage, including the ERK-1 [95, 96] and Notch/HES-1 [97] signaling pathways and intracellular calcium overload [98]. In addition, XPA and PARP1 appear to have regulatory interactions which would be upset in the XPA$^{-/-}$ cells[99]. These studies and their interpretation are complicated further by the observed cell-type specificity of PARP1 activation [96–98]. Resolution of these ambiguities rest, in part, on an elucidation of the cXBP cytosolic binding partner of XPA which sequesters this NER protein in the cytosol in normal G1 and S phases of the cycling cell and in the G_1/G_0 states of the non-cycling, highly differentiated cells. There are multiple possibilities since XPA has been described as a highly flexible scaffold protein capable of interacting with numerous proteins simultaneously [82, 89]. Future studies also are needed to elucidate XPA's possible cytosolic binding partner(s) in the G_1 and S phase cells, their biochemical properties, and possible normal function after XPA dissociation in G_2 and M phases.

Non-NER functions of XPA in the nucleus.

XPA functions as an essential component of the DNA damage repair complexes for both GG-NER and TC-NER. In addition, XPA binds to ds-ssDNA junctions with a significantly higher affinity (Kd = 49.1 ± 5.1 nM) [100] than it's specific binding to bulky DNA lesions (Kd = 200 nM) [101]. This suggests that, in addition to DNA damage recognition/verification, XPA may bind independently to and stabilize such ds-ssDNA junctions during the NER process and/or during other types of DNA metabolism. Hilton *et al.* recently demonstrated that in binding to ds-ssDNA junctions XPA employs a larger DNA-binding domain [102] than was previously described for repair substrates [103, 104].

How might this essential biochemical affinity for ds-ssDNA junctions relate to XPA's cytoplasmic restriction during S phase and XPA's performance of non-NER functions in cells? Hutchison-Gilford progeria syndrome (HGPS) patients suffer from a variety of laminopathy ailments due to a sporadic deficiency in the proteolytic processing of the precursor form of lamin A into the mature protein. The aberrantly processed protein produced is called progerin, a truncated form of lamin A with a hydrophobic farnesylated C-terminal [105–112]. HGPS cells with progerin accumulation exhibit a reduced replicative lifespan plus a deficiency in the repair of endogenous, laminopathy-induced DNA DSBs, which increase with age [113, 114]. These DNA metabolism deficiencies also correlate with a proteolytic truncation of replication factor C1 (RFC1) [115] and a sequestration of

proliferating cell nuclear antigen (PCNA) in a complex with progerin (Hilton *et al.*, private communication). Both the intact RFC complex and PCNA are essential replication factors and are needed for loading the replicative polymerase onto DNA [116, 117], thus accounting for the reduced replicative lifespan of HGPS cells [113, 118]. Interestingly, cellular nucleotide excision repair protein XPA misaccumulates at the DSB sites consisting of ds-ssDNA junctions even though XPA never has had a documented role in DSB repair, causing these breaks to become progressively devoid of DSB repair proteins [114]. Those DSBs appear to be generated from stalled and collapsed replication forks in HGPS. Depletion of XPA in these aging HGPS cells significantly relieves the deficiency in DSB repair, possibly by shifting the binding of available free PCNA to these XPA-free junctions (Hilton *et al.*, private communication). These observations suggest that as HGPS cells age progerin accumulates and sequesters PCNA, resulting in collapsed replication forks with DSBs and ds-ssDNA junctions to which XPA binds. Although this XPA binding may limit access to DNA DSBs repair proteins, it appears that the binding could stabilize the forks and prevent the HGPS cells from progerin-induced apoptosis (Hilton *et al.,* private communication). .

These potential non-NER roles allow for interesting speculation concerning XPA's pleiotropic functions and those of it's as-yet undescribed binding partners (i.e., cXBP) and will lead to many interesting experimental studies.

Conclusions

XPA is indispensable for both transcription-coupled repair and global genomic repair, and, thus, has a central and critical role in the NER process. Recent studies have revealed that XPA is kept in the cytosol in non-UV irradiated cells where it may be sequestered by a cytosolic XPA-binding protein, here termed cXBP. This subcellular distribution can be easily detected by immunofluorescence microscopy if the cells are fixed in cold methanol but not in cells fixed with *p*-formaldehyde. In the S phase UV irradiation induces a translocation of XPA into the nucleus for NER of UV-induced adducts. This S phase nuclear import is facilitated by XPA binding to by the transport protein importin-α4 (Fig. 2). In contrast, cells in G_1 phase retain XPA in the cytosol while XPA is mostly located in the nucleus in the G_2 phase; both the G_1 and G_2 phase distributions are largely independent of UV irradiation. Importin-α7 facilitates the G2 phase nuclear import of XPA. The S phase nuclear import of XPA is dependent on the kinase activity of ATR and on the tumor suppressor protein p53, which also is activated by the ATR kinase.

The ATR protein has multiple roles in regulating the NER process. In response to UV damage ATR regulates the NER process *via* its phosphorylation of numerous cell cycle control and DNA repair proteins. One of these is XPA; its phosphorylation by ATR is required for its essential role in NER of persistent CPD adducts. In addition, ATR kinase activity is required for the cytosolic-to-nuclear translocation of XPA by importin-α4 during S phase, the period when ATR kinase activity is at its highest. In addition to these kinase-dependent DDR nuclear functions a recent study reports an important cytosolic, kinase-independent role for ATR in moderating the intrinsic cell death response induced by UV irradiation. Surprisingly, newly formed ATR is a *cis*-conformer (ATR-H) at the Pro^{429} residue but the nuclear ATR is isomerized into the *trans*-isomer (ATR-L) by the proline

isomerase Pin1. It is likely that the prolyl isomerization of ATR may change the conformation of ATR between an unfolded structure to expose BH3 domain and a folded structure making BH3 inaccessible; the former is able to bind to and sequester the proapoptotic factor tBid at the mitochondrial surface to prevent initiation of the intrinsic apoptosis, thus allowing time for DNA repair.

XPA binds to ds-ssDNA junctions, such as those found at exposed replication forks and DNA regions undergoing repair. This binding, which is not necessarily unrelated to XPA's NER activity, is stronger than its binding to bulky DNA adducts. Prematurely-aging progeroid cells accumulate progerin, an abnormal form of lamin A and suffer from an accumulation of DNA DSBs and stalled replication forks. Interestingly, these sites are exposed due to sequestration of PCNA by progerin, allowing XPA to these DSB sites and stalled forks.

These studies have revealed several potential sites for therapeutic intervention to enhance the chemotherapy of cancer cells and/or the survival of progeroid cells.

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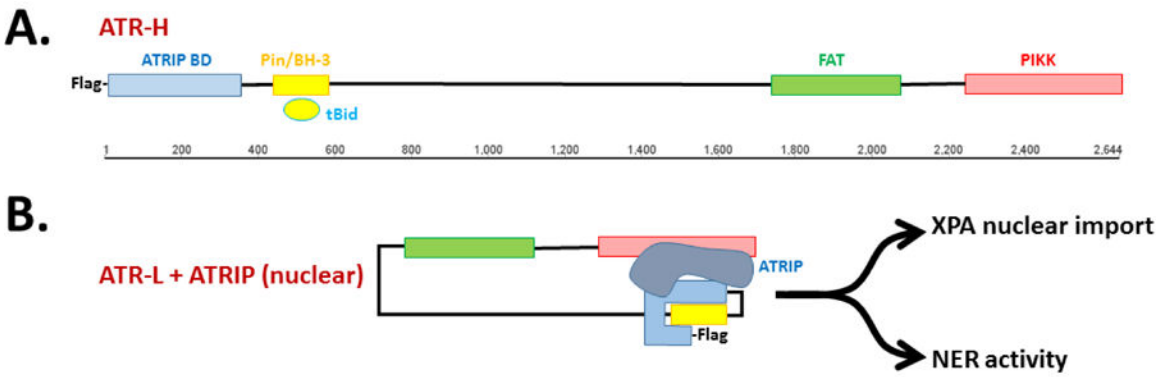

Figure 1. Possible alternative folding conformations of ATR-H vs. ATR-L.

There currently are no 3-demensional structures described for ATR. The diagrammatic representations presented here are based on the predictions of Hilton *et al.* for the N-terminal regions of ATR-H vs. ATR-L [40]. The N-terminal region of ATR-H, which has the *cis*-Pro^{429} isomer and an unphosphorylated Ser^{428}, is accessible to both tBid binding and to Flag antibody binding. Thus, ATR-H is presented in an open conformation. In ATR-L, which contains a phosphorylated Ser^{428} and a *trans*-Pro^{429}, the BH3 domain is inaccessible to tBid binding as is the Flag tag [40]. Thus, ATR-L is drawn with a folded N-terminal region. The N-terminus of ATR contains the ATRIP binding site; binding of ATRIP leads to activation of the ATR kinase *via* interaction with the C- terminal PIKK region [27–30]. Although speculative, the lower two diagrams of ATR-L illustrate this folding of the N-terminal region onto the C-terminal region, perhaps mediated by ATRIP binding.

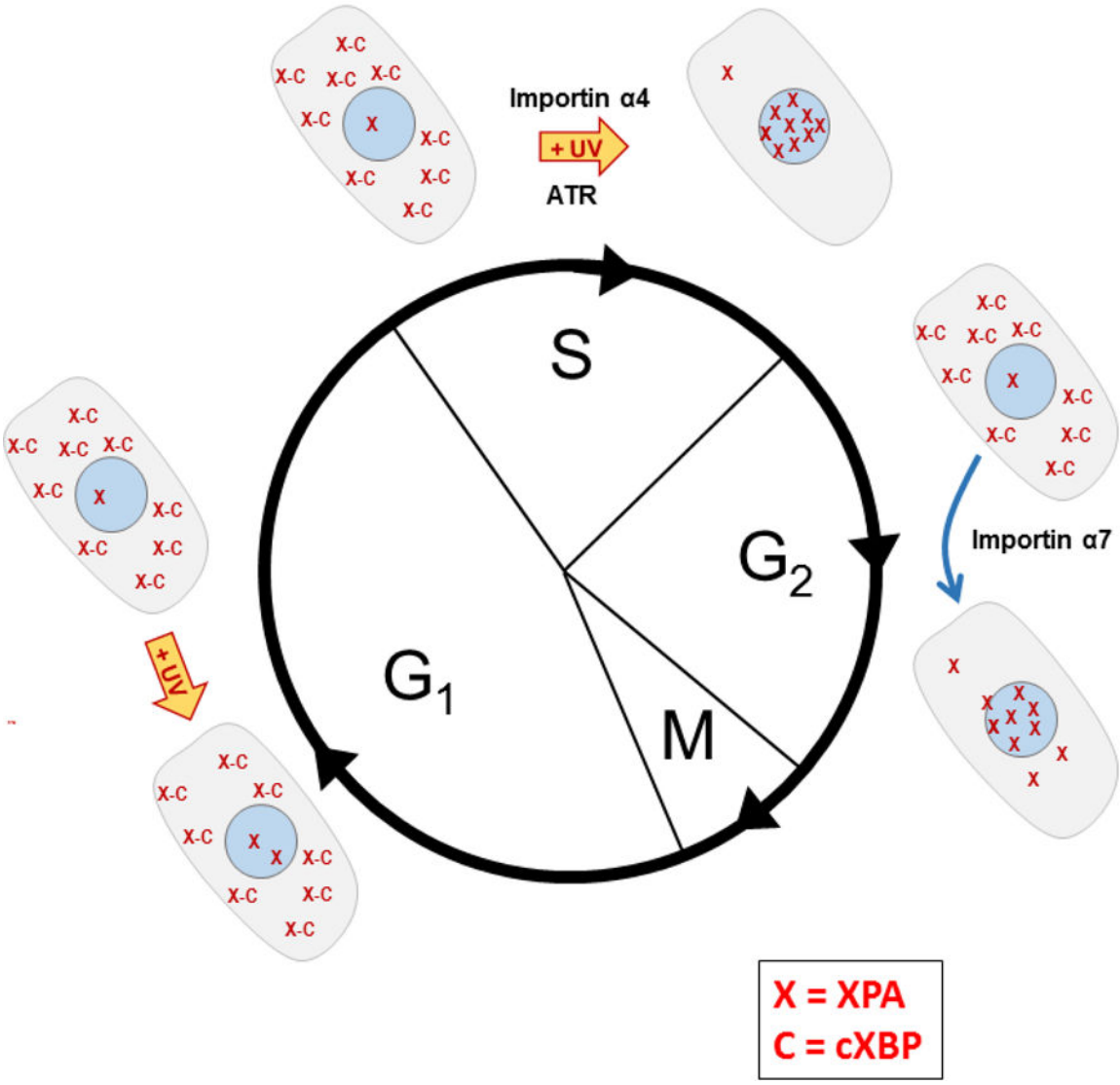

Figure 2. Normal and UV-induced redistribution during progression through the cell cycle in p53-competent human cells.

This model is based on the studies of Li et al. [68–70] In non-damaged cells in the G1 phase XPA (X) is mostly located in the cytosol, likely bound to cXBP (C), a hypothetical cytosolic XPA sequestration protein. Exposure of G1 cells to UV does not change this distribution. Likewise, in S phase cells XPA is mostly cytosolic; however, UV exposure induces a release of XPA from cXBP and a translocation of XPA into the nucleus. This XPA nuclear translocation in S phase requires the importin α4 transport protein and is ATR kinase- and p53-dependent in p53-competent cells. XPA is primarily located in the nucleus in G2 phase cells, transported there via importin α7 in a process independent of UV exposure. The XPA redistributes to the cytosol during the M-G1 phase transition and reassociates with cXBP.