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Review

Importance of PIKKs in NF-κB activation by genotoxic stress

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ABSTRACT

Alteration of the genome integrity leads to the activation of a vast network of cellular responses named "DNA damage response". Three kinases from the phosphoinositide 3-kinase-like protein kinase family regulate this network; ATM and DNA-PK both activated by DNA double-strand breaks and ATR activated by replication blocks. "DNA damage response" pathway coordinates cell cycle arrest, DNA repair, and the activation of transcription factors such as p53 and NF-κB. It controls senescence/apoptosis/survival of the damaged cells. Cell death or survival result from a tightly regulated balance between antagonist pro- and anti-apoptotic signals. NF-κB is a key transcription factor involved in immunity, inflammation and cell transformation. When activated by DNA double-strand breaks, NF-κB has most often a pro-survival effect and thereof interferes with chemotherapy treatments that often rely on DNA damage to induce tumor cell death (i.e. topoisomerase inhibitors and ionizing radiation). NF-κB is thus an important pharmaceutical target. Agents leading to replication stress induce a pro-apoptotic NF-κB. The molecular mechanisms initiated by DNA lesions leading to NF-κB nuclear translocation have been extensively studied these last years. In this review, we will focus on ATM, ATR and DNA-PK functions both in the IKKα/IKKβ/NEMO-dependent or -independent signaling pathways and on the regulation they can exercise at the promoter level of NF-κB regulated genes.

Contents

Abbreviations: aNHEJ, alternate non-homologous end joining; A-T, ataxia telangiectasia; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related kinase; cIAP-1, cellular inhibitor of apoptosis protein 1; cNHEJ, classical non-homologous end joining; CPT, camptothecin; DNA-PK, DNA-dependent protein kinase; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; DSB, double-strand breaks; Etp, etoposide; HR, homologous recombination; HU, hydroxyurea; IAP, inhibitors of apoptosis protein; IκB, inhibitor of κB; IKK, IκB kinase; IR, ionizing radiation; IRIF, ionizing radiation induced foci; NCS, neocarzinostatin; NEMO, NF-κB essential modulator; NF-κB, nuclear factor-κB; MRN, Mre11/Rad50/NBS; PARBM, poly(ADP-ribose) binding motif; PARP-1, poly(ADP-ribose) polymerase 1; PIASy, protein inhibitor of activated STAT y; PIDD, p53-inducible-death-domain containing protein; PIKK, phosphoinositide 3-kinase-like protein kinase; PP5, protein phosphatase 5; RPA, replication protein A; RIP1, receptor-interacting protein1; ROS, reactive oxygen species; SSB, single-strand breaks; TAK-1, TGF-beta activated kinase 1; WIP1, wild-type p53-induced regulator 1; XIAP, X-linked inhibitor of apoptosis protein.[2]

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1. Introduction

Genome integrity is continuously challenged. Lesions can arise during endogenous metabolism or following an aggression by external DNA damaging agents [1,2]. A large number of anticancer therapies (radiation therapies as well as chemotherapies) rely on genotoxic stress to induce the apoptosis of tumor cells. Regardless of their origins, exogenous or endogenous, accidental or intentionally induced, the cells respond to DNA lesions in a similar way and initiate an array of responses to minimize their consequences. Cellular responses prevent an excessive mutation load and favor survival, or, if the lesions are too numerous, induce cellular apoptosis to the benefit of the organism.

DNA damage response (DDR) is a collective name designing a network of intertwined signaling cascades initiated by replication stress and DNA lesions [3–5]. It includes not only cell cycle checkpoint activation, repair and transcriptional activation as previously thought but also encompasses control over the mRNA splicing machinery, the circadian circle and chromatin remodeling [6]. Some facets of the DDR are often deregulated in tumor cells, allowing them to escape apoptosis or senescence, and exacerbating the survival signals. The deregulation, by mutation or epigenetic silencing, can target upstream inducers, transducing kinases as well as lower effectors.

Lesion specific sensor proteins recognize the damage and initiate the DDR [4,7]. Three upstream serine–threonine kinases from the phosphoinositide 3-kinase-like protein kinase (PIKK) family regulate these cascades. The identity of the damage dictates the choice of the primary selected kinase. DNA-dependent protein kinase (DNA-PK) and ataxia telangiectasia mutated (ATM) are both activated by DNA double-strand breaks, "ataxia telangiectasia and Rad3-related kinase" (ATR) is activated by replication blockage.

Nuclear factor-κB (NF-κB) is a family of transcription factors that have a crucial role in inflammation, immunity, cell proliferation, development, survival and apoptosis. In human, the NF-κB family has five members: p65 (RelA) c-Rel, RelB, p50 and p52. Three of them (RelA, RelB and c-Rel) possess a trans-activating domain. NF-κB is induced by a variety of extra-cellular stimuli such as pro-inflammatory cytokines, bacterial lipopolysaccharides, viral RNA and DNA *via* the activation of membrane and cytosolic receptors [8,9]. It is also induced by low and high doses of ionizing radiation and numerous DNA damaging drugs [10–19]. Under these circumstances, the initiating signal originates from the nucleus. Indeed, it is absent in enucleated cells after topotecan (a DNA topoisomerase I inhibitor) treatment and is activated by restriction enzymes [12,20]. The kinetics of NF-κB activation by genotoxic stress are slow compared to the kinetics of activation initiated by inflammatory cytokines and the amplitude of the signal is less important [11,13]. NF-κB activation by aphidicolin and hydroxyurea (HU), two replication stress inducers, is even slower and weaker [21]. NF-κB activation is one of the numerous facets of the DDR cascade.

In most non-stimulated cells, NF-κB is maintained inactive in the cytoplasm by a family of inhibitors IκB. Some inflammatory cytokines activate the "classical" pathway that relies on the cytoplasmic IKK kinase complex and the ubiquitin- and proteasome-dependent degradation of the IκB inhibitors [22,23]. The core IKK complex contains two IκB kinases (IKKα, IKKβ) and a dimer of the regulatory subunit IKKγ (or NF-κB essential modulator (NEMO)). Once freed from its inhibitor, NF-κB moves to the nucleus where it positively or negatively regulates several hundred genes with the consensus κB binding site. The second main activation pathway, the "alternative" pathway predominantly active in B cells, is dependent on NF-κB-inducing-kinase, IKKα and the proteasome-dependent maturation of p100 in p52 [24,25]. Other minor pathways induced by UV radiation or oxidative stress are known [26–28]. In response to DNA damage, the best understood nuclear initiated cascades converge towards the "classical" pathway as they activate the IKK complex [12,13,29].

Cellular inhibitors of apoptosis protein (cIAP-1 and -2) and X-linked inhibitor of apoptosis protein (XIAP) are important regulators of NF-κB activation. Down-regulation of IAPs severely reduces NF-κB activation after genotoxic stress [30–32]. IAPs protect cells from genotoxic stress-induced apoptosis [33]. They are over-expressed in a variety of human cancers [34].

2. Genotoxic stress

DNA double-strand breaks (DSB) are the most severe genomic lesions. Incorrect repair induces chromosomal aberrations which can lead to loss of heterozygosity, pro-oncogenic fusion protein and cell death. DSB are responsible for tumorigenesis, neurodegeneration and premature aging. They are repaired either by homologous recombination (HR), classical non-homologous end joining (cNHEJ), back-up alternate NHEJ (aNHEJ) and single-strand annealing [4,35]. Most DSB are quickly repaired; only a fraction of them subsist longer such as those DSB initiating the DDR [36].

2.1. DSB induced by endogenous mechanisms

Endogenous DSB are directly formed during HR, V(D)J recombination in B- and T-lymphocytes and induced by intracellular reactive oxygen species (ROS) [37]. They are also indirectly formed by the collapse of stalled transcription forks or arrested replication forks. Replication fork arrest occurs during normal replication at sequences that are prone to secondary structures (i.e. tRNA genes and chromosomal fragile sites) [38,39]. Importantly, DSB are the potential end point of all DNA lesions. Indeed, incomplete repair process of any type of damage will *in fine* result in the creation of such breaks.

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2.2. DSB and replication stress induced by exogenous genotoxic agents

Low levels of genotoxic agents are present in our daily environment; higher levels are used in medicine as diagnostic tools or in the course of tumor therapies.

Ionizing radiations (IR) are source of many DNA lesions: base and desoxyribose oxidation, single-strand breaks (SSB) and DSB. SSB cluster, multiple SSB within 10–20 bp, gives rise to DSB with overhang. A dose of 1 Gy leads to approximately 1000 SSB and 20–40 DSB per cell [4]. Though minor in number, DSB are the most cytotoxic lesions after IR. Conventional radiation therapy consists in irradiating the patient repeatedly over several weeks with low doses (about 2 Gy/session) cumulating a total dose of 60–70 Gy. Presently, newest technologies (gamma knife) allow an extremely precise targeting of multiple radiation beams at the tumor and thus permit a much higher doses delivery and less repetitions. Ionizing radiations induce an important generation of ROS which are themselves involved in DNA damage formation. Diagnostic imaging techniques such as chest or dental X-rays and mammograms use much lower level of radiation and induce a small amount of DSB and oxidative stress.

Camptothecin (CPT) and its water soluble analogs topotecan and irinotecan are DNA topoisomerase I inhibitors used worldwide to treat cancer. CPT reversibly traps the open form of the cleavage complex "DNA/DNA topoisomerase I" creating SSB [40,41]. This drug affects both replication and transcription. During replication, the DNA polymerase collision with the "trapped cleavage complex" leads to a one end DSB [42]. This type of break is principally repaired by HR but not by NHEJ. During transcription, the stabilization of the "cleavage complex" by CPT interferes with the RNA polymerase II progression and generates an RNA–DNA loop that also promotes DSB [43,44]. Thus CPT induces single- and double-strand breaks in both dividing cells and non-dividing cells. Replication-dependent DSB require the ubiquitination of the trapped DNA topoisomerase 1 and its subsequent degradation by the proteasome [45]. Proteasome inhibitor also interferes with transcription-dependent breaks [46]. The number of DSB is higher in replicating cells. CPT toxicity is maximal in S phase. CPT effects are independent of an oxidative stress [11] (discussed in ref. [19]).

Etoposide (Etp), a non-intercalating DNA topoisomerase II inhibitor, prevents the re-ligation of the cleavage complex between "DNA/DNA topoisomerase II". It leads to DSB as well as SSB (7–20 SSB for each DSB). SSB arise if all monomers of the DNA topoisomerase II enzyme (a tetramer) are not simultaneously inhibited. It does not induce oxidative stress. Doxorubicin (trade name adriamycin) and daunorubicin are intercalating DNA topoisomerase II inhibitors that modify helical torsion. They induce equal amount of single- and double-strand breaks and an oxidative stress [14,41,47,48]. N-benzyl-adriamycin inhibits DNA topoisomerase II prior to the incision step and thus does not induce any breaks [49].

The nature of the break extremities is different in each situation described above. They will be recognized and processed by different enzymes in order to initiate signal transduction and generate re-sealable extremities.

Replication stress inducers like hydroxyurea (an antimetabolite), and aphidicolin (a reversible inhibitor of the DNA polymerase α) can be used alone or in combination with other drugs in the treatment of cancer, sickle cell anemia, and psoriasis. In normal cells, with an effective DDR, most arrested replication forks are stabilized and resolved, only a fraction of them collapses and leads to DSB.

3. Sensor complexes and proximal kinases

DNA double-strand break extremities are recognized and bound by MRE11/RAD50/NBS (MRN), Ku70–Ku80 complexes or PARP-1 within a few seconds of their formation.

3.1. MRE11/RAD50/NBS and ATM

MRN complexes bind to the break extremities, stabilize them close to each other, and, initiate DDR *via* NBS that recruits ATM kinase [50–52]. ATM main domains and features are given in Fig. 1 and extensively reviewed by Bhatti and co-workers [53–55]. In non-DNA-damaged cells, inactive ATM dimer associates with the histone deacetylase Tip60 through its FAT-C domain and with protein phosphatase 2A through multiple domains [56,57]. After DSB (induced by bleomycin or IR), MRN bound to the break extremities favors the recruitment of Tip60 on histone 3 trimethylated on K9. This interaction activates Tip60 that in turn acetylates ATM kinase on K3016 [58,59]. ATM acetylation induces then the autophosphorylation on S367, S1893, S1981 and S2996, and the dimer–monomer transition [60–62]. The release of protein phosphatase 2A that targets phosphorylated S1981 is required for the full activation of ATM [57]. Active ATM monomer has two nuclear localization sites. After irradiation and treatment by DNA topoisomerase inhibitors or neocarzinostatin (NCS, a radiomimetic drug), about 10% of ATM is associated with chromatin and integrated into the ionizing radiation induced foci (IRIF), structures that cover about 2 Mb on each sides of the break, whereas the majority of active ATM remains free in the nucleoplasm [5,63]. ATM activation by DSB is rapid and long lasting. Indeed, phosphorylated ATM is still detected several hours after the damage. It is activated by very low level of DNA damage (0.04 Gy) [60]. ATM, with its surprisingly large number of substrates (over 700), is the master regulator of the DSB-related DDR [6]. Its substrates, containing the consensus (S/T)Q target sequence, can be divided into subsets in function of their cellular localization: (i) associated with the chromatin (H2AX, Kap1) [64,66], (ii) integrated in the IRIF (MDC1, H2AX, 53BP1, BRCA1, NBS, MRE11, RNF20-RNF40) [5,65], (iii) phosphorylated by IRIF-bound-ATM but not themselves a part of these structures (Chk2) [67], (iv) phosphorylated by the nuclear free floating pool of ATM (p53, NEMO) [68,69], and, lately, a new subset of substrates localized in the cytoplasm (or at the plasma membrane) was defined (TAB2) [30]. Thus, after DNA damage, ATM phosphorylates numerous substrates both in the nucleus and in the cytoplasm, allowing a tight multilayered regulation of the downstream signaling cascades. With time, the IRIFs evolve and lead to the repair by HR in late S and G2 phase, or

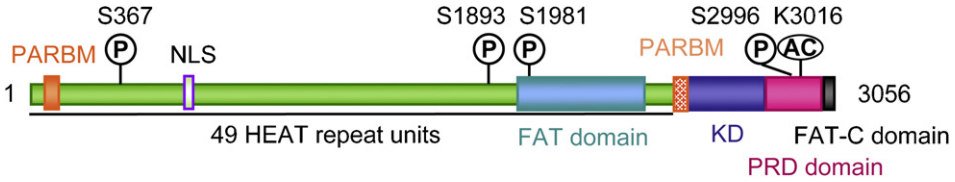

Fig. 1. Domain structure of ATM and IR-induced post-translational modifications. Schematic representation of the protein domains: FAT (FRAP/ATM/TRRAP) domain, KD (kinase domain), PRD (PIK regulatory domain) and FAT-C (FAT-C terminal domain). The three domains FAT, PRD and FAT-C regulate the kinase activity. The position of the 49 HEAT repeats (*h*untingtin, *e*longation factor 3, *A* subunit of protein phosphatase 2A and *T*OR1) [aa 1–2656]; the two PARBM (poly (ADP-ribose) binding motif) [aa 99–120, aa 2738–2760] and the NLS (nuclear localization signal) [aa 385–388] are indicated. After ionizing radiation K3016 is acetylated by Tip60 and four serine residues are autophosphorylated (S367, S1893, S1981 and S2996).

lead to the MRN-dependent alternate-NHEJ throughout the cell cycle [5,70].

Other proteins modulate ATM activation after DSB. For example, the mediator 53BP1 enhances ATM phosphorylation when the MRN complex is sub-optimal [71]. The serine/threonine protein phosphatase 5 (PP5) interacts with ATM and positively regulates its activation both after IR and NCS treatment [72]. The specific CPT-related ATM activation will be discussed in Section 4.1.1.

In human, ATM deficiency leads to ataxia telangiectasia (A-T), a multiphenotype disorder that reflects well the numerous tasks of ATM in DDR [73,74]. Affected individuals suffer from ataxia, ocular telangiectasia, inflammatory disease, insulin resistance, mental degenerescence, leukemias and lymphomas. A-T cells are hypersensitive to ionizing radiation, have a defect in their redox balance and do not exhibit cell cycle arrest after genotoxic injuries. They do not present a major defect in DSB repair. Only a subset of the DSB, localized in the condensed chromatin, requires ATM for repair [75].

3.2. Ku70/Ku80 and DNA-PKcs

Alternatively, Ku70/Ku80 dimers bind to the break extremities and recruit the catalytic subunit of DNA-PK (DNA-PKcs) to form the active DNA-PK [76]. DNA-PKcs, like ATM, is constitutively associated with Tip60 through its FAT-C domain. SiRNA against Tip60 reduces the phosphorylation of DNA-PKcs in response to bleomycin [77]. The activation of DNA-PK by CPT is more complex than after IR. Indeed, DNA-PK activation after CPT treatment requires replication and the 26S proteasome [78]. DNA-PK activation by IR is replication and proteasome-independent [78]. Active DNA-PK initiates the repair by cNHEJ (PARP-1- and MRN-independent). In human cells, the majority of the breaks are rapidly repaired by cNHEJ throughout the entire cell cycle [35,70]. Though it has a broader consensus target sequence than ATM, DNA-PK has a smaller number of known substrates. Most of them are implicated in the repair (itself, Artemis, PNK, XLF, ERCC4). DNA-PK autophosphorylation results in the inactivation of its kinase activity and its release from the Ku complex, two necessary steps for NHEJ completion. If the role of DNA-PK in repair is well understood, its role in signal transduction is less known. DNA-PK phosphorylates H2AX, KAP-1, p53, and signals to cell death machinery [79,80]. Specific inhibitors of DNA-PK have a lesser impact on DDR than specific inhibitor of ATM. The simultaneous addition of both inhibitors completely abolishes the cellular response after ionizing radiation [80–82].

Cells lacking DNA-PKcs are acutely sensitive to radiation and have gross repair defect. In human, severe combined immunodeficiency or SCID syndrome, a multigene associated disorder, is characterized by an absence of T lymphocytes. One sub-group is associated with a mutation in DNA-PKcs [83,84].

3.3. RPA and ATR

The replication stress sensor is replication protein A (RPA), a heterotrimer which binds to single-stranded DNA. RPA accumulates at stalled replication forks that generate long stretches of single-stranded DNA or at DNA repair intermediates. The accumulation of RPA permits the recruitment of ATR interacting protein (ATRIP) which interacts with the kinase ATR. ATR activation requires additional complexes and protein: Rad9/Rad1/Hus1 heterotrimer (loaded at the junction double/single stranded area by Rad17/RFC) and the DNA topoisomerase binding protein 1 (TopB1), an ATR activator [85]. PP5 is also a positive regulator of ATR [86]. ATR kinase activity is essential for stalled replication forks stabilization and restart, and for signaling to cell cycle checkpoint activation [87,88]. ATR targets the same consensus sequence and shares some but not all substrates with ATM. It is essential for viability and cell replication [89,90].

In humans, a splicing mutation affecting expression of ATR results in one form of the Seckel syndrome, a very rare multigene disorder with an altered ATR-controlled DDR characterized by intra-uterine growth retardation, dwarfism, microencephaly and brain anomalies [91,92].

3.4. Kinase relationship: distinct and cooperative functions

ATM, ATR and DNA-PK are primarily activated by a specific type of lesion as described above; however, the activation of one kinase does not exclude the activation of the other two. In fact, genotoxic lesions activate often more than one PIKK kinase, either simultaneously or sequentially. The collapse of arrested replication forks that activate at first ATR, will result in the phosphorylation of ATM [93]. It was shown that the Werner helicase is required for ATM activation after the collapse of the stalled replication fork induced by CPT or hydroxyurea [94]. And, during the processing of the DSB extremities, the Mre11- (and Exonuclease-1) dependent 5′-strand resection of DNA ends forms a 3′-single-stranded tail that is not only necessary to initiate HR but also to initiate the signaling to ATR [95]. In this situation, ATM activation precedes and is necessary for ATR activation [96,97]. CPT that induces both replication- and transcription-dependent DSB is known to activate the three kinases within a few minutes [98]. Moreover, cross-talks between kinases exist at the site of the break, i.e. ATM phosphorylates DNA-PKcs after IR, and DNA-PK controls ATM level [55,99,100]. ATR phosphorylates DNA-PKcs after UV-induced replication stress and is required to activate ATM after replication stress [93,101].

3.5. An additional sensor: poly(ADP-ribose) polymerase 1

Poly(ADP-ribose) polymerase 1 (PARP-1), a very abundant chromatin associated enzyme, is recruited at the level of both SSB and DSB within seconds. PARP-1 maintains genomic integrity and plays a role in base excision repair, SSB and DSB repair, DNA methylation, transcription, differentiation, chromatin structure and also signal transduction [102–104]. Once recruited to the breaks, PARP-1 is activated by post-translational modifications (PTMs) and adds poly(ADP-ribose) to acceptor proteins such as itself and histones. PARylation modifies the steric properties and the charge of the proteins, therefore changing their interacting partners. PARylated PARP-1, DNA bound or floating in the nucleoplasm, serves as docking platform for numerous proteins [105,106]. In a laser induced micro-irradiation study, PARP-1 was placed upstream of the rapid accumulation of the MRN complex at the site of the DSB [107]. Another study established by life imaging that PARP-1 recruitment at the site of the breaks is extremely fast (5 s) but transient. It is maximal 1–5 min after the breaks formation and completely gone at 5–15 min [106,108].

3.6. Negative regulation of the proximal kinases

The negative regulation of these kinases is less understood. Several phosphatases targeting them have been identified. The oncogenic serine/threonine phosphatase WIP1 (wild-type p53-induced regulator 1) negatively regulates ATM and reduces the phosphorylation of many proteins involved in DDR such as γH2AX, p53, Chk1 and Chk2. WIP1 directly binds ATM and dephosphorylates the residues S365 and S1981 [109–111]. The PP5 has a dual role. This phosphatase binds ATM, ATR as well as DNA-PKcs, but has a negative effect on DNA-PK and a positive one on ATM- and ATR-dependent cascades [72,86,112]. As indicated in Section 3.2, DNA-PK autophosphorylation represses it catalytic activity. In non-stressed cells, PP2A associates with ATM dimer and keep the

phosphorylation of S1981 low, thus contributing to maintain ATM inactive in basal conditions.

4. Signaling cascades coupling DNA lesions to NF-κB activation *via* the IKK complex

At first, several observations led to the conclusion that NF-κB is activated by DSB and not SSB or transcription blockage. NF-κB is activated by numerous DNA damaging agents that all have in common to induce DSB. In addition, CPT-elicited activation is maximal in S phase when the stalled replication forks are converted into DSB, and reduced if the replication is inhibited by aphidicolin [12,13]. Moreover, ATM dependency of NF-κB activation was established very early and that kinase is primarily activated by DSB as explained above.

4.1. ATM dependency

ATM is the main kinase involved in NF-κB activation following a genotoxic stress. Different studies determined how ATM controls this cascade both in the nucleus and in the cytosol. Table 1 summarizes the major milestones in unraveling its mechanism of action.

The first indication that ATM is required for this pathway was given in 1998 when Lee et al. observed that NF-κB activation by IR was reduced in A-T cells [10]. A year later, Piret et al. reported that, though a weak and transient NF-κB signal could be detected in some A-T cells after CPT, this signal was absent from other A-T cells and restored by complementation with ATM [113]. In 2001, ATM-dependent IKKβ activation after IR and NCS treatment in HEK293 cells was established [29]. These *in vitro* data were corroborated *in vivo*. Radiation-induced NF-κB activation was null in kidney and liver extracts from KO ATM−/− mice but easily detectable in the same tissues from WT mice. Transcriptomic analysis conducted with HEK293T cells revealed that ATM controls both NF-κB and p53-mediated NCS-induced transcriptional response [114].

4.1.1. Nuclear steps: NBS/MRE11/RAD50 is not always required for NF-κB activation by genotoxic stress

NF-κB activation by CPT and X-rays was investigated in cell lines derived from patients suffering from Nijmegen breakage syndrome and ataxia telangiectasia like disease lacking functional NBS or MRE11 respectively [115]. We observed that both NBS and MRE11 are required for NF-κB activation after X-rays (20 Gy) but not after CPT (10 μM), indicating that the CPT-related lesions do not require the MRN complex to activate NF-κB. Recent publications on ATM activation by CPT shed a new light on these results. Indeed CPT, contrarily to IR, induces a strong replication and transcriptional stress prior to the DSB which could lead to an alternate way of activating ATM [42–44]. During G1 phase or in non-dividing post-mitotic cells, ATM activation by CPT requires an active transcription [43]. The current hypothesis proposes that transcription fork stalling by the CPT-trapped DNA topoisomerase I generates DSB that in turn activate ATM. Corroborating these findings in other cells, Sakasai et al. also reported that transcription inhibition suppresses CPT-induced activation of ATM in HeLa cells, while replication inhibition does not [116]. This transcription-dependent activation of ATM is proteasome dependent [46]. It is not seen after Etp, IR or UV treatment. In another line of work, Takemura et al. compared ATM phosphorylation in CPT treated HCT116 (MRN deficient) cells and in the corrected isogenic cells re-expressing MRE11 (MRN proficient). They did not observe any difference [117]. Collectively these results and our data could indicate that ATM activation is MRN-independent after CPT treatment and thus explain why NF-κB activation by this compound is MRN-independent.

4.1.2. Nuclear steps: ATM phosphorylates SUMOylated NEMO

NEMO was identified as the regulatory subunit of the cytoplasmic IKK complex involved in the classical pathway. It has no enzymatic activity but its dimerisation is essential for the activation of the kinases IKKα and IKKβ. An additional small pool of free NEMO continuously shuttles in a CRM1-dependent manner between the nucleoplasm and the cytoplasm [118]. This pool of free NEMO is essential for the DNA damage mediated cascade and undergoes three sequential post-translational modifications: SUMOylation, phosphorylation and mono-ubiquitination (Fig. 2).

First, free NEMO is SUMOylated on K277 and 309 (>30 min after the damage) [119]. SUMOylation on these residues is typical of DNA damage signaling cascade. Indeed, it is not seen after cytokine treatment. PIASy (protein inhibitor of activated STAT y) is the SUMO E3 ligase that catalyzes SUMO-1 conjugation to NEMO

Table 1
ATM controls DSB-initiated NF-κB activation at multiple levels.

Year		Cell type\agent\dose	Ref.
General observations[a]			
1998	ATM is required for NF-κB activation	AT cells\IR\20 Gy	[10]
1999	ATM is required for sustained NF-κB activation	AT cells\CPT\10 μM	[113]
2001	ATM is required for IKKβ and NF-κB activation	HEK293\IR, NCS\20 Gy, 250 ng/mL *in vivo* tissues from KO or WT mice\IR\20 Gy	[29]
2005	ATM controls p53 and NF-κB transcriptional response	HEK293\NCS\200 ng/mL	[114]
Underlying molecular mechanisms			
Nuclear events			
2006	ATM directly phosphorylates $NEMO^{SUMO}$ on Ser 85	1.3E2, 70Z3, HEK293\Etp, CPT, Dox, IR\10 μM, 10 μM, 20 μM, 10 Gy	[69]
2009	A transient PARP-1/ATM/NEMO/PIASy complex is required for NEMO SUMOylation	MEFs PARP1−/−, NEMO−/−, PIASy−/−\IR\80 Gy HepG2\IR\30 Gy	[106]
Cytoplasmic events			
2010	ATM- and NEMO-dependent ELKS ubiquitination coordinates the assembly of TAK1/TAB2/3 and mono-ubiquitinated NEMO, mediating the IKK complex activation	HEK293, MEFS ELKS−/−, XIAP−/−, ATM−/−, TAK−/−\Etp, CPT, Dox\10 μM, 10 μM, 5 μM	[31]
2010	ATM, at the plasma membrane, promotes TAB2 accumulation (NEMO independent) ATM phosphorylates TAB2 (*in vitro*) on Ser 168	HepG2, HeLa\IR\10 Gy	[30]
2010	Cytoplasmic ATM triggers Ubc13-TRAF6-mediated poly-ubiquitination (proximal adaptor function) Identification of an ATM/TRAF6/cIAP1 module that catalyzes NEMO mono-ubiquitination in the cytoplasm	MEFs TRAF6−/−, PARP1−/−, PIASy−/−\IR\10 Gy	[30]

[a] Not an exhaustive list.

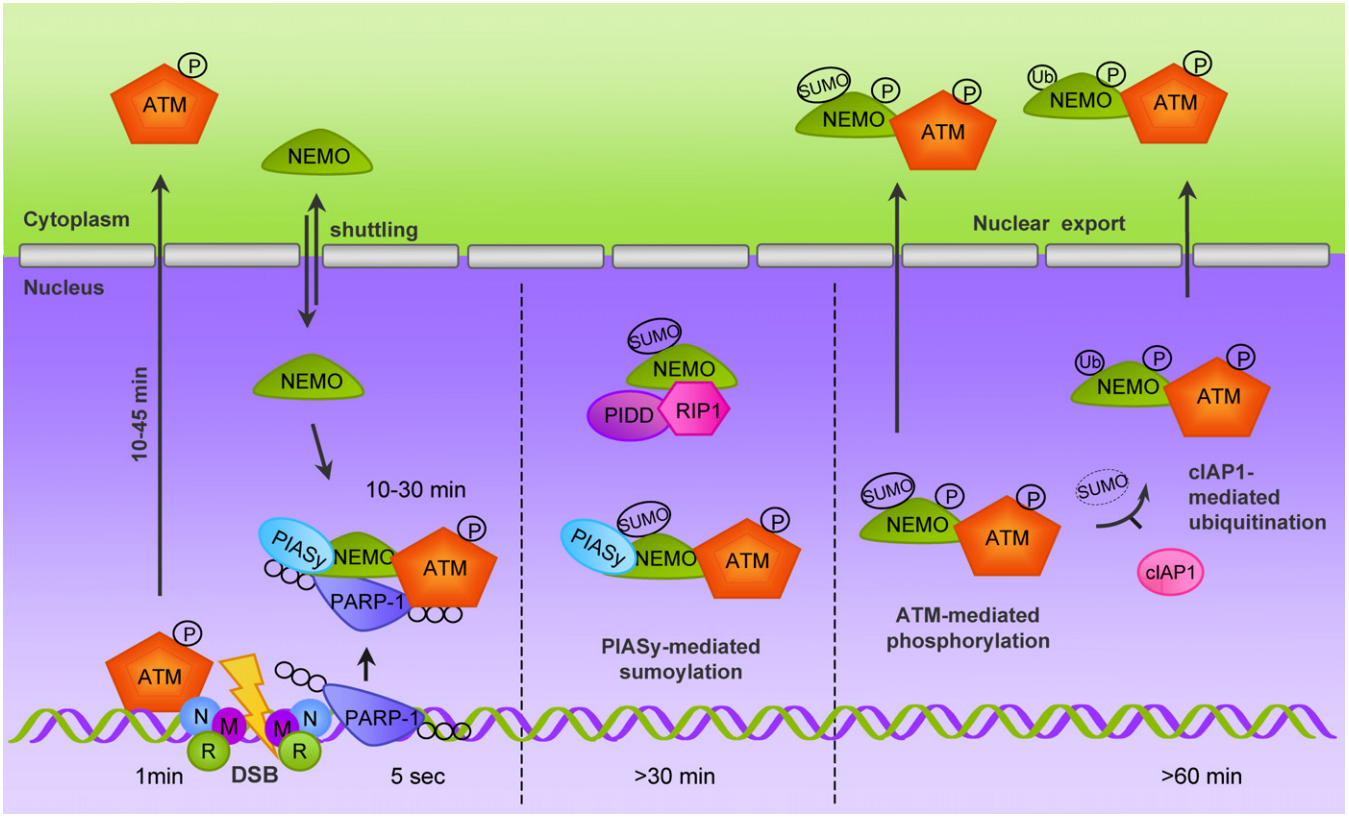

Fig. 2. Schematic representation of the nuclear steps of the cascade. Upon DSB formation, ATM and PARP-1 are rapidly activated and NEMO undergoes serial SUMOylation/phosphorylation/ubiquitination. The different complexes involved in these post-translational modifications are presented in function of time, the very transient PAR-ylated PARP-1 signalosome, the intermediate PIDD/RIP/NEMO complex that enhances (in some cells) the PIASy mediated SUMOylation and the later events leading to NEMO phosphorylation by ATM and its mono-ubiquitinisation by c-IAPS. The two scenarios for the cytoplasmic export of SUMOylated NEMO or mono-ubiquitinated NEMO are represented. Additional details are given in the text.

[120]. Two different protein complexes modulate PIASy activity in this context.

After DNA damage, PIDD (p53-induced death domain protein) and RIP1 (receptor interacting protein 1) associate with nuclear NEMO in a heterotrimer and accumulate in the nucleus. The events upstream PIDD nuclear translocation are not yet completely elucidated. This PIDD/RIP1/NEMO complex assists NEMO SOMUylation after CPT and Etp treatments in HEK293 cells [121]. RIP1 assures the connection between NEMO and PIDD. RIP1 requirement for NF-κB activation in Mouse embryonic fibroblasts (MEFs) after CPT- and IR-related lesions had been reported prior to this work [122]. NEMO SUMOylation is ATM-independent. NEMO–RIP1 complex plays a role both at low and high level of damage [123].

The second modulator of NEMO SUMOylation identified is PARP-1. Stilmann's team established that PIASy binding to active PARylated-PARP-1 is required for NEMO SUMOylation in MEFs and HepG2 cell lines [106]. They showed the existence of transient nuclear signalosome containing PARylated- PARP-1, ATM, NEMO and PIASy. This complex is detected between 10 and 30 min after DSB formation and is not attached to the chromatin at the site of the lesion. ATM, like PIASy, contains a PAR binding domain (PARBM) allowing its anchorage to PARylated-PARP-1 [106,124]. In contrast, NEMO binds directly to the non-PARylated form of PARP-1. As this signalosome brings the E3 SUMO ligase and NEMO in close proximity, one would assume that the SUMOylation takes place there. Nevertheless, the story is likely more complicated as (i) kinetic studies suggest that NEMO SUMOylation by PIASy takes place after the disappearance of this specific signalosome, and (ii) the PIASy PAR binding motif covers partially the RING finger-like domain essential for PIASy E3 activity. Thus PIASy has to dissociate from the signalosome before acting. Neither PIDD nor RIP1 are associated with the PARP-1 signalosome, indicating that PIDD/RIP1 could either have a role downstream of the PARP-1 signalosome or its requirement be cell type dependent.

On the contrary, other groups reported that DNA damage signaling to NF-κB can be PARP-1-independent. Indeed, PARP inhibitors do not have any effect on p50/p65 nuclear translocation in human breast cancer cell lines or MEFs after IR (20–50 Gy) and in CEM after CPT, Etp or daunorubicin treatment [11,125].

Subsequent to its SUMOylation, NEMO is phosphorylated by ATM on S85 [69]. It is possible that PARP-1 bound ATM phosphorylates SUMOylated NEMO, but this was not investigated. The first PAR binding motif of ATM is located in N-terminal (aa 99–120), therefore binding to PARylated PARP-1 through this motif would not interfere with its kinase activity [124]. However, since ATM possesses a second PARBM (aa 2738–2760) that overlaps with the kinase domain, binding through this second motif could eventually interfere with its enzymatic activity. This second PARBM is less evolutionary conserved and was not investigated. What is certain is that NEMO is not recruited to the IRIF indicating that the phosphorylation takes place away from this structure. NEMO phosphorylation is the convergence point between two distinct signaling cascades: the first one very rapid leading to ATM activation and the second one somewhat slower leading to NEMO SUMOylation either PIDD or PARP-1 supported.

Third, cIAP-1, a nuclear E3 ubiquitin ligase, mediates NEMO subsequent mono-ubiquitination at K277 and 309 [32]. cIAP-1 competes with E3 SUMO ligase PIASy for NEMO modification as both bind to the same region of NEMO and target the same residues. When over-expressed, cIAP-1 inhibits NEMO SUMOylation. This ubiquitination is dependent of the phosphorylation by ATM. No deSUMOylase has been identified yet.

4.1.3. From nucleus to cytoplasm

The molecular events necessary for the nuclear export of NEMO, essential to convey the signal from the nucleus to the cytoplasm where the IKK complex resides, are partially elucidated.

One group reported that nuclear NEMO mono-ubiquitination is required for its Ca^{2+} and probably Ran-GTP-dependent nuclear export. Both ATM and NEMO are exported together [119,126]. While a second team observed NEMO mono-ubiquitination in the cytoplasm after the transport of SUMOylated NEMO in this compartment. This team detected a Ca^{2+}-dependent but PARP-1/NEMO/PIASy-independent ATM export [30].

4.1.4. Cytoplasmic steps: cytosolic ATM is required for IKK activation

In order to activate the IKK complex, ATM plays additional roles in the cytoplasm, some of them independent of NEMO mono-ubiquitination (Fig. 3).

TGF-beta activated kinase 1 (TAK1) is a kinase upstream of the IKK complex in the classical pathway. After cytokine treatment, TAK1 activates the IKK complex by phosphorylating directly the S177/181 of the IKKβ subunit [127]. TAK1 associates with TAB1 or TAB2 at the cytoplasmic membrane. Two research teams have shown that ATM promotes TAK1-dependent IKK activation after DNA damage either with TRAF6 or ELKS requirement [30,31].

The first team reported a nuclear export of phosphorylated ATM into the cytoplasm 10–45 min after irradiation. This export is independent of PIASy, NEMO or PARP-1, and is Ca^{2+} dependent. This cytoplasmic pool of ATM has two functions. First, ATM recruited to the plasma membrane promotes TAB2 accumulation and probably phosphorylates it on S168 (positive *in vitro* kinase assay) allowing TAK1/TAB2^{P} cytoplasmic release. Second and independently, cytosolic ATM binds to TRAF6 and triggers TRAF6 UBC13-mediated auto-K63-polyubiquitination. To fulfill this last function ATM acts as an adaptor and not as a kinase. Auto-ubiquitinated TRAF6 then recruits TAK1/TAB2^{P}, NEMO and cIAP1. cIAP1 E3 ubiquitin ligase catalyzes the mono-ubiquitination of NEMO on S285. The close proximity of TAK1 proteins stimulates its trans-autophosphorylation. Together, these two events lead to IKK activation.

These data show that cytosolic ATM functions both like a kinase and like a proximal adaptor [30]. ATM nuclear export is independent of PARP-1 and of SUMOylated NEMO. On the opposite, the mono-ubiquitination of NEMO by cIAP-1 requires the export of SUMOylated NEMO. IKK activation thus requires the convergence of three different cascades.

The second team reported that TAK1 activation by CPT, IR and Etp requires the K63-linked-polyubiquitination of ELKS by XIAP (another E3 ubiquitin ligase) in an ATM- and NEMO-dependent manner. ELKS (ELKS a protein rich in glutamine, leucine, lysine and serine) was previously reported to bind NEMO and is associated with IKK. It acts upstream of the IKK activation and downstream of

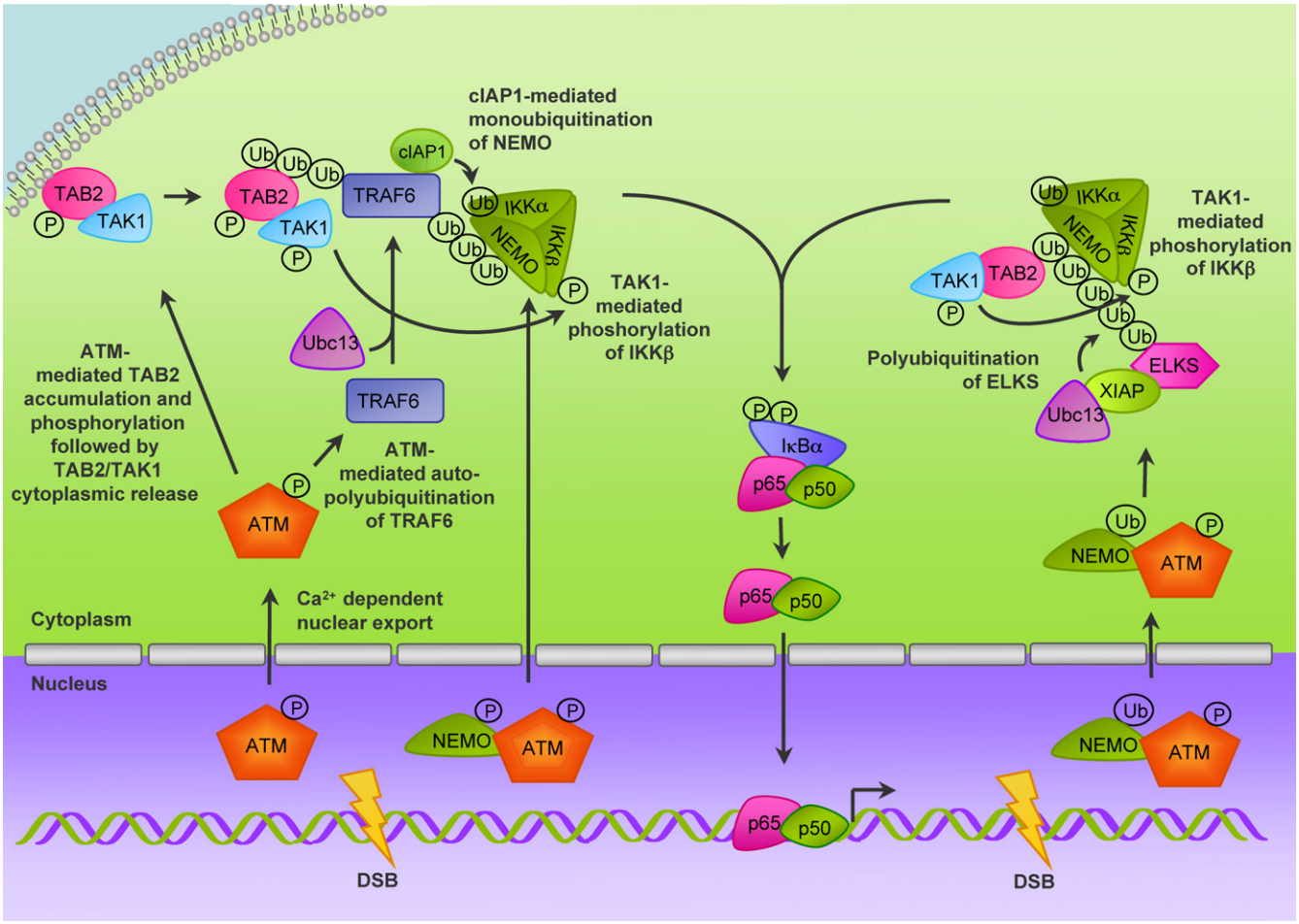

Fig. 3. Schematic representation of the cytoplasmic steps of the signaling. The two identified cascades, TRAF6 or ELKS mediated, leading to IKK complex activation in the cytoplasm are represented. In the situation depicted on the left of the figure, cytosolic ATM has two functions one as a kinase and the second as a proximal adaptor. NEMO ubiquitination is mediated by c-IAP and IKKβ phosphorylation by active TAK1. In the situation depicted in the right part of the figure, ATM-dependent TAB2-TAK1-IKK activation is represented. Both poly-ubiquitinated TRAF6 or ELKS serve as docking platforms to favor the proximity of TAB2-TAK1 and IKKα/β/NEMO complex. In the middle of the figure, the IκBα phosphorylation and ubiquitination will initiate its proteasome-dependent degradation allowing the nuclear transclocation of p50/p65.

ATM [69]. Poly-ubiquitinated ELKS serves as a docking platform to TAB2,1/TAK1 and IKK allowing the phosphorylation of IKKβ. TAK1 activation is dependent of NEMO phosphorylation and subsequent nuclear export. According to this team, NEMO mono-ubiquitination is required for nuclear export.

4.1.5. Cytoplasmic steps: IκBα degradation

The subsequent steps of the signaling cascade are identical to the steps induced by pro-inflammatory cytokines. Active IKKβ phosphorylates the IκBα inhibitor on S32 and 36, these phosphorylations trigger the K48-linked ubiquitination on K21 and 22 by the E3-ubiquitin ligase Skp1-Cul1-F-Box complex. Ubiquitinated IκBα is then targeted for selective degradation by the 26S proteasome allowing p50/p65 to move to the nucleus. The addition of 26S proteasome inhibitors (ALLN or lactasystin) prior to CPT treatment leads to the accumulation of phosphorylated poly-ubiquitinated IκBα and the down-regulation of NF-κB [12,13,17,19].

4.2. ATR dependency

ATM and ATR have opposite effect when it comes to genotoxic stress-induced NF-κB.

Only one study was conducted directly linking NF-κB activation with replication stress inducers primarily activating ATR. It showed that hydroxyurea and aphidicolin activate a delayed and weaker NF-κB response when compared to Etp [21]. Although replication stress inducers primarily activate ATR, the authors observed that NF-κB activation induced by replication stress is still dependent of ATM, PIAsy, NEMO SUMOylation/phosphorylation/ubiquitination, IKK activation and IκBα degradation. In brief, it is identical to the DSB-mediated cascade described in Section 3.1.

Interestingly, ATR down-regulation by specific siRNA increases NF-κB transcriptional response in untreated cells as well as in cells treated by HU, aphidicolin, IR or Etp. These observations indicate that active ATR behaves as a negative regulator of NF-κB in both basal conditions and stress conditions. The molecular mechanism underlying this observation was investigated. Both ATM and ATR bind NEMO through their FAT domain and compete to interact with the same domain of NEMO, however only ATM phosphorylates NEMO on S85. Thus activated ATR represses ATM-dependent NF-κB activation. Interestingly, ATM and ATR bind the N-terminal NEMO sequence that is required for its interaction with IKKβ. Additionally, the repression of ATR by siRNA is likely to destabilize the stalled replication forks and increase the number of DSB.

4.3. DNA-PK dependency

Different teams investigated Ku and DNA-PKcs requirement for NF-κB activation after genotoxic stress. Experiments, conducted in DNA-PK proficient (MO59K) or deficient (MO59J) glioblastoma revealed that NF-κB nuclear translocation after CPT treatment is not modified [113]. Opposite results were obtained by Basu et al. who demonstrated that DNA-PK was essential to nuclear translocation of NF-κB after irradiation (10 Gy) using the same cell lines [128]. Yet, a few years later, it was shown that liver and kidney extracts prepared from DNA-PKcs−/−, Ku70−/− or Ku80−/− mice irradiated (20 Gy) still activate IKKβ indicating that after IR, DNA-PK is not required for this signal generation [29].

A DNA-PK-dependent activation cascade leading to IKKβ activation through the mitogen-activated protein kinase $p90^{sk}$, independently of ATM, was reported after a treatment with N-benzyl-adriamycin, an inhibitor of DNA topoisomerase II. However, this drug inhibits DNA topoisomerase II prior to the incision step and thus could initiate an alternate signaling cascade as no DSB are created [129].

5. Signaling cascades coupling DNA lesions to NF-κB activation through an IKK-independent mechanism

DNA-damage-induced processing of p100 to p52, the hallmark of the alternative pathway, was investigated by Perkins' team. The phosphorylation of p100 on S866, a prerequisite for this processing, was observed after CPT, cisplatin, Etp, UV and IR treatment in osteosarcoma. The phosphorylation was IKKβ-independent and IKKα-dependent as expected, but surprisingly it was also NEMO and ATM dependent. The feeding of p100 could result from the classical activation pathway but not its phosphorylation on S866. Further investigations need to be conducted as, in the "typical" alternative pathway, IKKα activation is NEMO-independent. If confirmed, it would be interesting to identify the post-transcriptional modifications of NEMO involved in this new function [130].

A novel mechanism of NF-κB activation, involving nitration and independent of the cytoplasmic IKK complex, was described by Yakovlev et al. They observed that IR (2–8 Gy) induces a rapid nitration of IκBα on Y181 and Y305 by the NO synthase in CHO-K1 and MCF breast cancer cells. The Y181 nitration leads to the dissociation of an intact IκBα from NF-κB. The mutation of this residue prevents the NF-κB activation [131].

6. Additional levels of control by PARP-1, DNA-PK and ATR over NF-κB activation

PARP-1 controls NF-κB activation at two levels; firstly, upstream of the IKK activation (see Section 4.1.2), and secondly, at the promoter site as a co-activator that regulates the expression of inflammatory target genes. This second function is not restricted to genotoxic stress. PARP-1 localizes to the promoter of almost all actively transcribed genes including p50/p65 regulated pro-inflammatory genes and regulates transcription [102,132,133]. Both p50 and p65 interact directly with PARP-1. PARP-1 has different modes of action. In a first study, after irradiation of MCF7, Veuger et al. have shown that a PARP-1 inhibitor does not affect p50/p65 nuclear translocation, but delays *ikba* mRNA resynthesis [125]. That result was not corroborated when MEFs PARP-1−/− were compared to MEFs PARP-1+/+ [125]. In another study, non PARylated PARP-1 was shown to bind to p50 therefore preventing its recruitment to the promoter [134].

DNA-PK can also control NF-κB after its binding to κB sites at specific promoters. It phosphorylates RNA polymerase I and II as well as the transcription factors c-Jun, Oct-1, and NF-κB [79,135–137]. For example, after TNFα treatment DNA-PK positively regulates the κB-dependent expression of the adhesion protein VCAM-1 by phosphorylating p50 bound to this specific promoter. The phosphorylation increases both the stability of the p50/p50 or p50/p65 dimers and their binding to the promoter. In absence of DNA-PK, TNFα still induces the degradation of IκBα and the nuclear translocation of p50/p65 but not the transcription of the *VCAM-1* mRNA, indicating that this phosphorylation is essential for the transcription. DNA-PK activation after TNFα treatment could be related to oxidative stress [136]. DNA-PK was also found to phosphorylate the inhibitor IκBα both in N- and C-terminal. These phosphorylations are thought to increase IκBα binding to NF-κB and thus reduce its activity at the basal level [138].

An indirect effect of ATR was reported. Indeed, after cisplatin treatment, the kinase Chk1 activated by ATR phosphorylates p65 on T505. This phosphorylation leads to the transcription repression of the anti-apoptotic factor Bcl-X_L [139].

7. IKKε and NF-κB regulation after genotoxic stress

IκB kinase ε (IKKε or IKKi), a member of the IKK family, is a known breast cancer oncogene over-expressed in breast cancer,

ovarian cancer and glioma [140–142]. IKKε is not integrated in the IKK complex [143]. It is though to regulate late NF-κB target genes [144] and is able to phosphorylate IκBα (on S36 only) as well as p65 [145,146]. IKKε over-expression contributes to cisplatin resistance in ovarian cancer and to doxorubicin resistance in glioma [141,142]. In a recent publication, Renner et al. reported that upon Etp treatment, the IKKε contributes to the pro-survival function of NF-κB. They demonstrated, that IKKε translocates into the nucleus after the induction of the DNA lesion and associates with the PML nuclear bodies where it is SUMOylated. This SUMO-modification allows IKKε to phosphorylate p65 on S468. The over-expression of wild-type IKKε in MEFs KO for IKKε enhanced survival while the over-expression of a mutated form of IKKε that cannot undergo SUMOylation does not [147]. These data establish a link between IKKε and the DNA-damage-induced NF-κB.

8. ATM and the oxidative stress

In response to ROS, an active disulfide-cross-linked dimer of ATM was recently identified and characterized [148,149]. The C2991 residue present in the FAT-C domain of ATM is essential for this DSB- and MRN-independent activation of the kinase. This active ATM dimer shares some substrates (i.e. p53) with the classical active ATM monomer but not all (i.e. KAP1) [149]. Additionally, a SUMOylation of NEMO similar to that observed after DSB was also detected after oxidative stress [20]. As ionizing radiation and many DNA damaging drugs induce simultaneously an oxidative stress and DNA breaks it will be important in the future to verify "if" and "how" this active ATM dimer affects the DSB-elicited NF-κB cascade. Can it phosphorylate SUMOylated NEMO? Does it favor IKK activation in the cytoplasm? If the answer to these questions turns out to be positive, then ATM could exercise an additional level of regulation to this already complex cascade.

9. Functional consequences of NF-κB activation by genotoxic stress

NF-κB targets both pro-apoptotic and anti-apoptotic genes. The cell survival depends of the expression ratio between the genes belonging to these two classes.

In the case of genotoxic stimuli such as Etp, CPT or IR, NF-κB activation leads to increased cell survival, as following TNFα treatment. It acts as an inducer of anti-apoptotic genes such as Bcl-xL, XIAP, ciap1, ciap2 and survivin. The protection role of this factor against DNA-damage-induced apoptosis has been linked to increased resistance of tumors to chemotherapy and is well documented [12,13,17,19,150,151]. NF-κB is thus an important therapeutic target. Two key steps of the signaling cascade, the IκB-kinase and the 26S proteasome have been mainly targeted, and, specific inhibitors developed. The inhibitors of the IκB-kinase are reviewed elsewhere [152]. Bertezomid (trade name Velcade, also known as PS-341), is an FDA approved proteasome inhibitor, used to treat multiple myeloma. Bertezomid acts in synergy with radiation and chemical drugs such as doxorubicin, CPT-11 and irinotecan [153–156]. Other compounds of lesser toxicity have also promising effects [157]. It would be simplistic to believe that the effects on viability, observed when proteasome inhibitors are added, are solely related to the absence of degradation of IκBα and the subsequent down-regulation of NF-κB. Indeed, the proteasome controls the degradation of many other proteins important for DSB repair that alter the fate of the damaged cells too [158]. ATM itself, the master regulator of the DDR, is an essential therapeutic target. Three selective inhibitors are known: KU-55933, CP466722 and KU-60019 [159–161]. The specific inhibitor KU-55933 has a low intrinsic cytotoxicity and is currently under clinical trial. It acts as a molecular switch that transiently inhibits ATM kinase activity. KU-55933 increases the cytotoxicity of ionizing radiation, CPT, Etp and doxorubicin [159]. NF-κB down-regulation plays a role in this increase mortality.

However, the effects of NF-κB on cellular outcome after genotoxic stress are not so clearly defined. Indeed, several reports have shown that the functional consequences of NF-κB activation differ depending on the cellular context and the nature of the inducer. For example, in U-2 OS cells NF-κB activation induced by daunorubicin, doxorubicin, UV-C and mitoxantrone leads to a repression of anti-apoptotic target genes whereas these same genes are activated in response to Etp in the same cell line [14,162]. Actually, DNA topoisomerase II inhibitors that also intercalate into DNA induce repression of NF-κB target genes whereas the others activate them [14]. The molecular mechanism of this repressor potential of NF-κB is not well understood. On one hand, repressor potential of NF-κB in response to cisplatin is due to post-translational modification of p65 that leads to recruitment of co-repressors [139] whereas on the other hand doxorubicin treatment induces decrease of post-translational modifications of NF-κB, leading to repression of its target genes [163]. In other cases the molecular mechanism is unknown yet.

Some other reports have also shown that NF-κB activation is even required for DNA-damage-induced apoptosis in some contexts. Indeed, MEFs p65−/− cells are more resistant to cell death induced by UV and Etp, compared to the p65 rescued cells. Noxa, a pro-apoptotic Bcl2 family member, apparently regulated by p73 in this context, was shown to play a critical role in this p65-dependent process [164]. Apoptosis induction in glioblastoma by different genotoxic agents (doxorubicin, daunorubicin and mitoxantrone) was also shown to depend on NF-κB. Indeed, in these cells, inhibition of NF-κB, by siRNA against p65 or by over-expression of IκBα-SuperRepressor, leads to a decrease of DNA-damage induced apoptosis. Importantly, this NF-κB mediated apoptosis is not due to repression of anti-apoptotic genes by this transcription factor (as in U-2 OS cells). In this case, we have a transcriptionally active form of NF-κB induced by genotoxic intercalating agents that leads to apoptosis. This pro-apoptotic function of NF-κB is p53-independent [165]. However, in another cancer cell line such as neuroblastoma, NF-κB activation by Etp and doxorubicin has no effect on cell survival [166]. So final outcomes of NF-κB activation by genotoxic agents is really context dependent.

DNA-damage-induced activation of alternative NF-κB pathway plays an important role in apoptosis induction too. Indeed, a pro-apoptotic effect of p52 has been observed after both UV-C and cisplatin treatment [130]. As in the classical pathway, in response to these two genotoxic stimuli, p52 represses anti-apoptotic genes and, furthermore, it cooperates with p53 to induce expression of p53-dependent pro-apoptotic genes (PUMA, DR5, Bax, Gadd45α).

NF-κB activation by replication stress inducers is also pro-apoptotic. 1.3 E3 cells (NEMO deficient) re-expressing NEMO-S85A are more resistant to HU in a clonogenic assay than 1.3 E3 cells re-expressing NEMO-WT. Numerous pro- and anti-apoptotic genes are expressed after HU and Etp treatments at similar or different levels. *Bcl-X_L* mRNA is up-regulated by Etp not by HU. Chromatin immunoprecipitation analyses confirmed the similar recruitment of p65 on the promoter but revealed that CBP/p300 was recruited on the promotor after Etp treatment whereas HDAC1 was recruited after HU, explaining the differential transcription rate and the different survival effect [21].

All of these points are therefore to be considered for the development of combined chemotherapeutic strategies using NF-κB inhibitors.

10. Concluding remarks

NF-κB activation by genotoxic stress results from the convergence of many distinct cascades both in the nucleus and in the cytoplasm. It is probably why it is slow and variable from one cell line to another. Today the overall picture of the molecular mechanism underlying this signal transduction is better understood than yesterday but it still remains incomplete and somewhat confused. We have to keep in mind that this cascade is studied with damage induced by different genotoxic agents, at different doses and in different cell types probably adding to the complexity of this picture.

Though ATM is without any contest the master regulator of NF-κB activation by genotoxic stress (where it plays at least three different roles), the two other kinases have important functions too. They control the level of NF-κB activation and affect its pro-survival or pro-death functionality. In some other facets of the DDR cascade, ATR can substitute for ATM and phosphorylates the same substrates with the same final outcome. It is not the case for DSB-elicited NF-κB activation. On the contrary, ATR blocks the ATM phosphorylation of NEMO which is an essential step for NF-κB activation after DNA lesion. In this respect, ATR role is particularly significant. Here too, it is important to keep in mind that (i) ATM and ATR expression levels vary from cell lines to cell lines and from tumors to tumors [21] (http://www.ebi.ac.uk/gxa/), and, (ii) ATM and ATR are activated with different kinetics and ratio in function of the nature of the lesion and in function of the cell cycle phase. There are few (or no) situations where a single kinase is activated except when the other kinases are mutated or non-expressed. Even drugs with a single cellular target, like CPT, initiates parallel signaling cascades that affect each other.

The cytosolic ATM adaptor function is a new concept. ATM, like the other PIKK kinases, is a giant protein with a large N-terminal HEAT repeat domain (49 HEAT repeats are found in ATM). These repeats are important for protein/protein interaction and have structural properties [54,167]. This domain covers a large portion of the protein (85%), the kinase and the regulatory domains (PRD and FAT-C) account only for 15% of the total protein. ATM can bind simultaneously to many proteins and hold them in vicinity of each others. A rigid heat repeat domain could have a purely structural role like the heat repeat domain of DNA-PKcs that forms a ring around the DNA extremities and gathers repair proteins at the site of the break before phosphorylating them [167]. A more flexible HEAT repeat domain like in pR65 could be assimilated to a moving scaffold that accommodates around proteins in function of, for example, their PTMs [168]. The presence of active ATM in the cytoplasm indicates that once it has been activated by DSB, ATM might act away from the DNA, in this regard it differs from DNA-PK that is always DNA bound when it acts as a kinase.

The nuclear translocation of p50/p65 is only one aspect of the NF-κB activation, numerous p50/p65 PTMs control the identity of the target genes, the nature of the co-factors recruited and the signal termination. While a lot is known on the p65 PTMs after cytokine treatments, less is known on p65 PTMs after DNA damage [169–171].

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