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NF-κB activation by double-strand breaks

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Abbreviations:
AT, Ataxia Telangiectasia
ATLD, Ataxia Telangiectasia-Like Disease
ATM, Ataxia Telangiectasia Mutated
ATM^P, phosphorylated ATM
ATR, ATM- and Rad 3-related kinase
CC, coiled-coil domain
CPT, camptothecin
DNA-PK, DNA–protein kinase
Dox, Doxorubicin
DSB, double-strand breaks
HED, hypohydrotic ectodermal dysplasia
IκB, inhibitor of κB
IKK, IκB kinase
IR, ionizing radiation
NBS, Nijmegen Breakage Syndrome
NCS, neocarzinostatin
NEMO, NF-κB essential modulator
NF-κB, nuclear factor-κB

ABSTRACT

Cellular response to DNA damage is complex and relies on the simultaneous activation of different networks. It involves DNA damage recognition, repair, and induction of signalling cascades leading to cell cycle checkpoint activation, apoptosis, and stress related responses. The fate of damaged cells depends on the balance between pro- and antiapoptotic signals. In this decisive life or death choice, the transcription factor NF-κB has emerged as a prosurvival actor in most cell types. As corollary, it appears to be associated with tumorigenic process and resistance to therapeutic strategies as it protects cancerous cells from death. In this review, we will focus on NF-κB activation by double-strand breaks inducing agents, such as ionizing radiation and DNA topoisomerase I and II inhibitors routinely used in cancer therapy. Coinciding with the 20th anniversary of the NF-κB discovery, major steps of the DSB-triggered cascade have been recently identified. Two parallel cascades are necessary for NF-κB activation. The first one depends on ATM (activated by double-strand breaks) and the second on PIDD (activated by an unknown stress signal). The phosphorylation of NEMO by ATM is the point of convergence of these two cascades. The identification of ATM/NEMO complex as the long searched ''nuclear to cytoplasm'' signal leading to IKK activation is also a major piece of the puzzle. The knowledge of the precise steps leading to DSB-initiated NF-κB activation will allow the development of specific blocking compounds reducing its prosurvival function.

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MRN, Mre11/Rad50/NBS
PIDD, p53-inducible death domain-containing protein
RIP, receptor-interacting protein
ROS, reactive oxygen species
SSB, single-strand breaks
ZF, zinc finger

1. Introduction

Genotoxic stress, imposed by chemical or physical exogenous and endogenous agents, constantly challenges the integrity of the genome. To cope with this permanent danger, cells have developed a large array of responses. Upon double-strand breaks (DSB) detection, fine tuned networks of signalling pathways are initiated leading to (i) breaks repair by homologous recombination or non-homologous end joining, (ii) checkpoints activation that induces a sudden arrest of the cell cycle at the G1/S or G2/M border or within the S phase, (iii) death by apoptosis mostly *via* p53, (iv) activation of mitogen activated protein kinase and transcription factors AP1 and NF-κB [1–5]. These different networks share common steps and are coordinated. Though DSB are relatively rare in a normal environment, they are extremely cytotoxic and lead to chromosomal aberrations, genetic instability and eventually cancer or cell death if not correctly processed. DSB may result from administration of genotoxic drugs such as DNA topoisomerase I and II poisons or exposition to ionizing radiation (IR) used to eradicate cancer cells through induction of apoptosis. Whatever their origins, accidentally or intentionally induced, cellular responses to DSB are identical.

Camptothecin (CPT) and its clinically used derivatives (Irinotecan/CPT-11 and Topotecan) are DNA topoisomerase I inhibitors, whereas the epipodophyllotoxin Etoposide/VP16 and the anthracycline Doxorubicin (Dox/Adriamycin) are DNA topoisomerase II inhibitors [6,7].

In this review, we will exclusively develop the DSB-induced NF-κB response. NF-κB activation pathways initiated by other DNA damaging agents, i.e. ultraviolet radiation A and B are described elsewhere [8–11].

2. The NF-κB family

Discovered 20 years ago as a nuclear protein that binds the enhancer of the kappa immunoglobulin-light chain in B cells [12], the NF-κB family of transcription factors is now regarded as ubiquitous factors regulating immune and inflammatory responses, development, apoptosis, tumour development and progression [13–20]. It is activated by a large variety of stimuli: cytokines, bacterial lipopolysaccharide, phorbol myristoyl acetate, virus, oxidative stress, ultraviolet and ionizing radiation and genotoxic drugs [21]. NF-κB positively or negatively affects the expression of its target genes [13,22].

NF-κB are dimers of five proteins: p50, p52, p65 (RelA), c-Rel and RelB. Only three of them (namely p65, c-Rel and RelB) have a transactivating domain, p50 and p52 do not. The heterodimer p50/p65 is the most common form encountered, but all members of the family can associate to form homo- or heterodimers [13]. The nature of the dimer will dictate its transcriptional activity on each promoter. In a resting state, NF-κB dimers exist as a latent form and shuttle between the nucleus and the cytoplasm. Their predominant cytoplasmic localization results from an equilibrium state influenced by interaction with a family of inhibitors (IκBα, IκBβ, IκBγ, IκBε, Bcl-3 and the precursor proteins p100 and p105) that mask their nuclear localization signal and thus maintain them mostly in the cytoplasm [13,23,24].

3. DSB are different chemical entities

Topoisomerase inhibitors and IR used to eradicate cancerous cells generate different types of DSB. DSB generated by CPT result from the collision of the replication fork with the stabilized cleavage complex "DNA topoisomerase I/DNA/CPT" and, therefore, occur exclusively during the S phase of the cell cycle [6,25]. The stabilized cleavage complex is generated throughout the cell cycle as DNA topoisomerase I relieves torsional stress during DNA replication and transcription. The 3′ extremity of the break is linked to the enzyme through a phosphotyrosine bound and the 5′ end is free (5′-OH) [6,26]. VP16 and other DNA topoisomerase II inhibitors stabilize the covalent enzyme-cleaved DNA complex mostly throughout S, G2 and M phases when the enzyme is upregulated. DNA toposisomerase II influences chromosome dynamics during DNA replication, recombination, chromosome condensation and segregation and also transcription [27]. DSB induced by topoisomerase II inhibitors are limited by a 5′ end linked to the enzyme through a phosphotyrosine bound and a 3′-OH end. Finally, DSB in irradiated cells are the major cytotoxic lesions but they represent only 2% of the total breaks and often result from clusters of single-strand breaks (SSB). The nature of the extremities are either deoxyribose fragments or –phosphate or –OH groups [1,28].

4. DSB are the nuclear signal initiating the NF-κB activating cascade

In addition to DSB, topoisomerase I and II poisons and IR also generate reactive oxygen species (ROS) that could trigger the NF-κB cascade independently of DNA damage. Evidence that NF-κB activation by these agents finds its origin in DSB and not in ROS or another exclusively cytoplasmic signal came from multiple observations: (i) CPT does not generate detectable ROS in conditions that activate NF-κB [29], (ii)

N-acetylcystein (an antioxidant molecule) does not interfere with the activation of NF-κB by CPT but perturbs H_2O_2 signalling [29], (iii) NF-κB activation by CPT is maximal if the drug is added on S phase synchronized cells corresponding to DSB generation time and minimal during G1 phase when only SSB are present [30,31], (iv) Etoposide fails to activate NF-κB in enucleated cells [31] contrasting with phorbol myristoyl acetate or UV radiation that remain fully capable of activating NF-κB in cytoplasts [10,31], (v) active forms of DNA topoisomerases I and II enzymes are required for NF-κB activation by topoisomerases poisons [31,32] and finally (vi) the Ataxia Telagiectasia Mutated protein kinase, an intermediate essential to this signal transduction (see Section 6.1), is not activated by ROS [28]. Taken together, these observations provide evidence that nuclear DSB are the initiating point of the cascade.

5. The molecular mechanism leading to NF-κB activation by DSB is atypical

The cascade leading to NF-κB activation elicited by DSB differs from both classical or alternative pathways initiated by cytokines [8,13,16,33–35]. It also diverges from other non-conventional pathways induced by UV radiation [8–11,36] or oxidative stress [37–42].

As a reminder, in the classical pathway triggered by the proinflammatory cytokines (TNFα, IL1β), the cytoplasmic IκB kinase complex (IKK) is activated. This complex includes IKKα and IKKβ kinases associated to the scaffold protein NF-κB essential modulator (NEMO) also named IKKγ [43,44]. This leads to the phosphorylation, by the IKKβ subunit, of the IκBα inhibitor on two N terminal S32 and S36 residues. The inhibitor is subsequently ubiquitinated on K19 and K21 and degraded *via* the 26S proteasome pathway [13,33]. The alternative pathway, triggered by the cytokine Lymphotoxin β or the B-cell activating factor, is dependent on the kinase IKKα only and on NF-κB inducing kinase [16,35]. Both classical and alternative pathways are reviewed elsewhere in this issue.

In the following paragraphs, we will describe the best-characterized DSB-induced NF-κB response. This pathway is common for CPT, VP16 and IR. However, it is not unique, some DNA topoisomerase II inhibitors act differently. For example, Dox shares many aspects of the common DSB-induced cascade (see below) but initiates an alternate ATM-MEKK-ERK cascade and IKK-independent pathway [45,46]. Dox is in fact a multi-featured weapon, that alters DNA in more than one way. It effectively creates DSB when it inhibits topoisomerase II but it also intercalates into DNA, thereby modifying the helical torsion, and generates DNA adducts that are known to mediate cell death in a non-topoisomerase II related fashion [22,47]. It is therefore not surprising that the initiated cellular cascades differ from a strictly DSB signalling.

The intensity of NF-κB activation for a given level of DSB varies with cell types and transformation status. This is especially true for IR, as it was observed that transformed cells respond better than non-transformed cells [48].

6. The cascade: mechanistic insight

6.1. *ATM initiates the nuclear cascade*

Three large evolutionary conserved nuclear S/T kinases belonging to the family of the ''phosphoinositol-3-kinase (PI3K)-like protein kinase'' regulate the cellular cascades initiated by genotoxic stress: Ataxia Telangiectasia Mutated (ATM), ATM- and Rad 3-related kinase (ATR) and the catalytic subunit of DNA protein kinase (DNA PKcs) [3,49–52]. These enzymes are the initial kinases activated in the cellular response to DNA damage but do not sense it directly and need specific partner proteins [53].

At low level of DNA damage, within doses used in radiotherapy, ATM activation is regulated by the heterotrimer Mre11/Rad50/NBS [53,54]. Mre11/Rad50/NBS binds to DSB extremities and carries out multiple functions including DNA repair (both homologous recombination or non-homologous end joining) and cell cycle checkpoint activation [55]. ATR, essential for viability, requires RPA and ATRIP to bind to single-strand DNA. DNA-PKcs requires the heterodimer Ku70–Ku80 to bind to DSB extremities. Ku70 and Ku80 are two non-essential proteins tightly associated that recognize and bind free DNA ends. Bound Ku70 and Ku80 recruit and activate the catalytic subunit DNA-PKcs. The active complex, named DNA-PK, is essential for non-homologous end-joining repair of DSB induced by IR or created during V(D)J recombination in B cells [56]. Cells deficient in Ku70 and Ku80 are hypersensitive to IR. Other roles of DNA-PK in telomere length maintenance, signal transduction, apoptosis and innate immunity are known and reviewed elsewhere [50].

Deficiency in ATM leads to a pleiotropic recessive human disorder called Ataxia Telangiectasia (AT) characterized by cerebellar degeneration, immunodeficiency, radiation sensitivity, chromosomal instability and also cancer predisposition [3,57]. Two closely related but distinct syndromes are the Nijmegen Breakage Syndrome (NBS) and the Ataxia Telangiectasia-Like Disorder (ATLD) that express mutant forms of NBS and Mre11, respectively [58–60]. Cells derived from AT, ATLD and NBS patients share an increased sensitivity to IR and DSB-generating compounds [55].

In resting conditions, ATM is kept inactive under a dimeric form in the nucleus. In presence of DSB, ATM dimer dissociates and one molecule phosphorylates the adjacent molecule on S1981 (Fig. 1). Active phosphorylated ATM (ATM^P) is detected within minutes of the DSB formation and is stable for many hours [61]. At low dose IR or treatment with the radiomimetic Neocarzinostation (NCS), ATM autophosphorylation is Mre11/Rad50/NBS-dependent [54,62,63]. In presence of DSB, but in absence of Mre11, a non-phosphorylated ATM monomer can be observed [64]. Experiments with recombinant proteins confirmed the requirement of both Mre11/Rad50/NBS complex and DSB for optimal phosphorylation of ATM [65]. ATM^P has two different sub-nuclear localizations, some molecules are found in the vicinity of the DSB and are integrated in the IR-induced foci while most molecules move freely in the nucleoplasm. Is a very transient recruitment of all ATM molecules to DSB site necessary for its activation or is the activation propagated from close to close? That question still needs clarification. Importantly, chromatin-bound and free

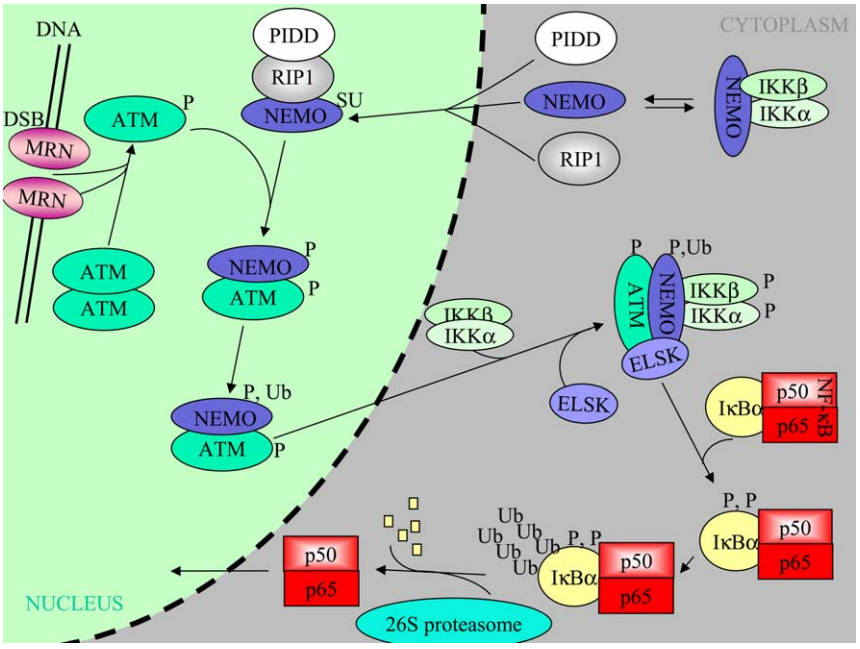

Fig. 1 – General scheme of DSB-elicited NF-κB activation. DSB trigger two independent cascades that converge to NEMO to activate NF-κB. In one cascade, ATM is rapidly activated by DSB (in a Mre11/Rad50/NBS (MRN)-dependent manner when few lesions are generated). In the other cascade, activated PIDD translocates into the nucleus where it associates with RIP1 and NEMO. The complex enhances NEMO sumoylation. The two cascades converge when ATM binds to and phosphorylates NEMO allowing its subsequent mono-ubiquitination. Modified NEMO (phosphorylated and mono-ubiquitinated), still bound to ATM, leaves the nucleus and associates with IKKα and IKKβ. The recruitment of ELSK is necessary for the optimal activation of this atypical canonical IKK complex. Activated IKK phosphorylates IκBα and allows its subsequent poly-ubiquitination that will target the inhibitor for proteosomal degradation. Unbound NF-κB (mostly p50/p65 dimer) is then free to move in the nucleus and regulates its numerous target genes.

ATM^P target different substrates. H2AX, NBS, 53BP1, MDC1, BRCA1 are phosphorylated in the IR-induced foci structure, p53 is phosphorylated by free ATM^P [66].

AT cells are hypersensitive to DSB-generating agents [3]. Many teams have presented evidence that IKK and NF-κB activation are ATM-dependent after IR, CPT, VP16 and Dox treatment in transformed cells [45,67–69]. That dependence seems to be partial in some primary AT cells as a weak and shortened NF-κB activation can be detected in such cells [69].

The role of NBS and Mre11 proteins in the signalling cascade initiated by CPT and X rays has been investigated with cell lines derived from NBS and ATLD patients. It was observed that NF-κB activation by X rays was attenuated and delayed in NBS deficient cell lines and absent from Mre11-deficient cell lines [70]. Surprisingly, neither NBS nor Mre11 had a role in the NF-κB activation by CPT. This could mean that Mre11/Rad50/NBS is only required for ATM activation after irradiation and not after CPT treatment. Alternatively, the experimental conditions could have been such that the DSB threshold below which Mre11/Rad50/NBS is required for ATM activation was overcame after CPT treatment and not after irradiation.

The participation of ATR to the NF-κB cascade initiated by DSB is not reported. Downexpression of ATR by siRNA fails to attenuate the level of NF-κB after CPT treatment in HEK293 cells [67].

DNA-PK does not play a role in the activation of NF-κB after IR [68] or CPT [69]. The topoisomerase II inhibitor, *N*-benzyladriamycin activates a MEK/ERK/$p90^{rsk}$/IKK pathway in a DNA-PK-dependent and ATM-independent manner but this drug inhibits the enzyme before the cleavage and thus, does not induce DSB [45].

6.2. *PIDD is essential to relay a stress signal*

PIDD (p53-induced protein with a death domain) was identified as a death domain-containing protein inducible by p53 and promoting apoptosis. It also contains leucin rich repeats in its N-terminal portion [71]. In resting conditions, PIDD continuously shuttles between the cytoplasm and the nucleus [72]. Upon genotoxic stress, its spatial repartition is altered and it partially accumulates in the nucleus. In 2004, Tinel et al. have identified a first PIDD complex necessary for apoptosis. In this PIDD/RAIDD/caspase 2 complex, RAIDD is an adaptor protein assuring the recruitment and activation of caspase 2 after treatment with VP16 or Dox [73]. Recently, a second PIDD complex essential for NF-κB activation by genotoxic stress was identified. This NF-κB-related complex contains PIDD/RIP1/NEMO [72] and accumulates in the nucleus after genotoxic treatments (Fig. 1). The role of RIP1 (receptor-interacting protein 1) is to assure the recruitment of NEMO to PIDD. The importance of RIP1 in this cascade was previously known but its position in the pathway had remained elusive [74].

The exact nature of the stress signal leading to the transfer of PIDD into the nucleus and the subsequent recruitment of RIP and NEMO is still unknown. This signal could originate in the nucleus and either leave it to activate PIDD in the cytoplasm or directly activate nuclear PIDD. Once stably formed, the PIDD/RIP/NEMO complex enhances the sumoylation of NEMO, in a yet undetermined manner [72].

6.3. *NEMO is the point of convergence of the two cascades*

NEMO is a 48 kDa acidic polypeptide unusually rich in glutamic acid and glutamine (13% each) able to form multimers. Its different domains are represented in Fig. 2. The coiled-coil domain 1 (CC1) is necessary for the interaction with IKKα and IKKβ kinases [75], CC2 mediates its oligomerization [76,77] and the leucine-rich (LZ) domain is critical for nuclear export [78]. The function of the zinc finger (ZF) is presented in the following paragraphs.

Two human syndromes are associated with defect in NEMO, whose gene is located on the X chromosome [79]. The first one, classical Incontinentia pigmenti, leads to granulocytic infiltration of the skin in female and death in males [80]. It is observed when one copy of NEMO is missing. The second one is a X-linked primary immunodeficiency characterized by an hypohydrotic ectodermal dysplasia (HED) as well as a hyperproduction of IgM. It results from point mutations or splicing mutation in the ZF domain [81–84].

NEMO was first shown to have a cytoplasmic role as the structural component of the classical IKK complex essential to the formation of the large 700–900 kDa complex [43,85,86]. Though NEMO has no enzymatic activity by itself, its presence and dimerisation are essential for the kinase activity of IKKα and IKKβ. The association of NEMO with IKKα and IKKβ is dynamic and can easily be challenged with peptide identical to the IKK-interacting domain [75]. NEMO is also necessary for the connection of the IKK classical complex to the upstream signalling activators at the level of membrane receptors [13].

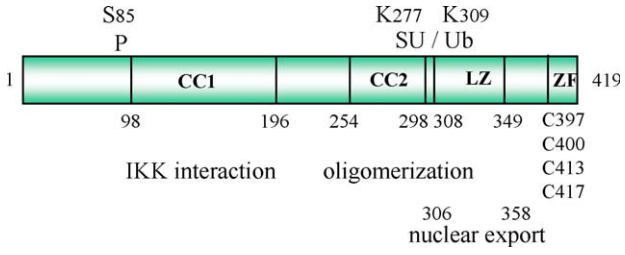

Fig. 2 – Schematic representation of NEMO. The different domains and their function are indicated. The N-terminus large coiled-coil motif (CC1) is necessary for the interaction with IKKα and IKKβ kinases. The C-terminal half contains a second coiled-coil motif (CC2) that mediates its oligomerization, and a leucine-rich (LZ) domain critical for nuclear export, and the zinc finger (ZF) composed of four cysteine residues. ZF is necessary for DSB-related sumoylation. The amino acids modified by phosphorylation, sumoylation and ubiquitination are indicated. SU, sumoylation sites; P, phosphorylation site, Ub, ubiquitination sites.

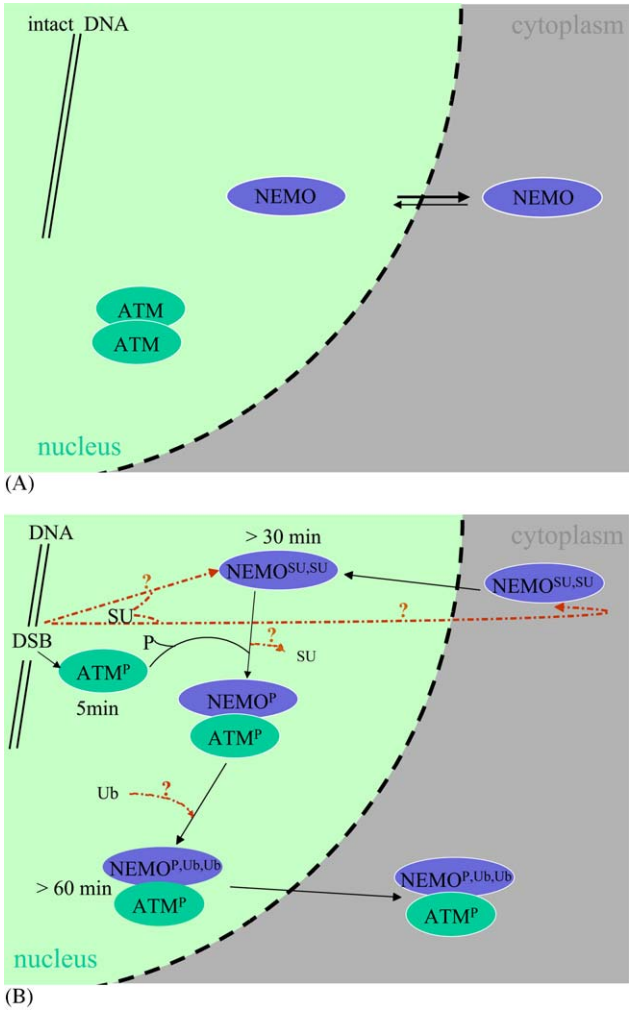

Fig. 3 – Subcellular localization of NEMO depends of its modifications. (A) *NEMO localization in resting conditions.* NEMO unattached to IKK shuttles between the cytoplasm and the nucleus. The highest concentration is found in the cytoplasm. ATM dimers are inactive in the nucleus. (B) *Spatio-temporal localization of NEMO and ATM after DSB detection.* ATM is rapidly activated upon DSB detection. NEMO, slowly sumoylated on K277 and K309 by an unknown stress signal, accumulates in the nucleus. The subcellular localization of the NEMO during its sumoylation is unknown. Nuclear $NEMO^{SU}$ is then bound and phosphorylated by active ATM and subsequently mono-ubiquitinated. SUMO peptides are removed by an hydrolase before the ubiquitin addition. NEMO phosphorylated on S85 and ubiquitinated on K277 and K309 leaves the nucleus with activated ATM. Black solid arrows indicate the identified steps; brown dashed arrows the unidentified steps. The time indicates the early detection of the modified species.

In resting conditions, free NEMO (like PIDD) constitutively shuttles between the cytoplasm and the nucleus in a CRM1 dependent mechanism (Fig. 3A) [78]. NEMO apparent function in the nucleus of non-stressed cells is to repress NF-κB regulated genes by interacting with CBP [78].

In addition to these roles in resting conditions or after cytokine stimuli, NEMO plays an essential role in DNA damage-mediated responses. Initial observations revealed that cells lacking NEMO do not activate NF-κB after DNA damage. Further studies identified the specific residues and domains of the protein involved. The first 120 AA (necessary for interaction with the IKK kinases) are necessary for both DNA damage or cytokine signalling cascades, whereas the ZF domain is important for the cascade initiated by weak activators such as topoisomerase inhibitors (CPT and VP16), and to a lesser extent for the cascades initiated by fast and strong activators such as LPS [87,88]. Furthermore, point mutations found in HED (C417R/D406V) perturb DNA damage signalling to NF-κB, indicating that ZF is absolutely essential to DNA damage signalling [87].

The precise role of ZF became clear when it was established that it is indispensable for NEMO sumoylation on K277 and K309 [67] (Fig. 2). This modification allows the accumulation of an IKK-unbound form of NEMO in the nucleus without affecting its stability (Figs. 1 and 3B). The sumoylation of NEMO is exclusively observed with DNA-damaging compounds and never found with cytokines. The disruption of the two sumoylation sites (K277, K309) abrogated the DSB dependent IKK activation. As indicated in the previous section, the sumoylation is enhanced if NEMO is part of the PIDD/RIP/NEMO^{Su} complex [72]. The specific events leading to the sumoylation are ATM-independent and remained unidentified [67].

Nuclear NEMO^{Su} is then phosphorylated on S85 by active ATM^{P} (Figs. 1 and 3B) [89]. This phosphorylation probably takes place in the nucleoplasm independently of the IR-induced foci as NEMO does not seem to be recruited to these foci after genotoxic stress [89]. The importance of the S85 was confirmed for CPT, VP16, IR and Dox but not for LPS or TNFα.

Subsequent to this phosphorylation, NEMO^{P} is mono-ubiquitinated by yet unidentified E2 and E3 ligases (Fig. 3B). It was noted that NEMO^{P} was desumoylated in an ATM-independent manner prior to its mono-ubiquitination and that the modified lysines are identical for both modifications [67]. The ubiquitination of NEMO marks it for nuclear export. Additionally, coimmunoprecipitation studies have revealed that ATM^{P} is taken along into the cytoplasm. The heterodimer ATM^{P}/NEMO^{P,Ub} joins with IKKα and IKKβ, and together form an active IKK complex (Fig. 1) [89]. According to these findings, ATM^{P}/NEMO^{P,Ub} would be the long searched nuclear to cytoplasm signal leading to IKK activations by DSB. Is there an exchange of NEMO protein with a pre-existing IKK complex or is it newly formed? This has not been investigated yet.

Thus, there is a fine tuned spatiotemporal control of NEMO regulated by different posttranslational modifications (Fig. 3B). Kinetic analyses have shown that ATM is activated within minutes of DSB formation, whereas NEMO sumoylation peaks later after 45–60 min and NEMO ubiquitination after 60–90 min. This indicates that NEMO sumoylation is the limiting step of the cascade [67].

6.4. *Activation of cytoplasmic IKKα/β/NEMO/ATM complex and downstream events*

Early experiments with knockout *IKKα*$^{-/-}$, *IKKβ*$^{-/-}$ and *NEMO*$^{-/-}$ cells have revealed that the three subunits of the classical complex are indispensable for DSB-induced cascade (CPT, IR, NCS) [11,31,68]. With our actual knowledge of the molecular mechanism of this cascade, we can assert that this complex is, in fact, slightly different from the classical complex activated by cytokines as it contains ATM (Fig. 1).

ELKS was first identified as a regulatory subunit of the classical IKK complex essential to its activation after TNFα treatment [90]. It was established that ELKS helps in the recruitment of IκBα and thereby facilitates its phosphorylation and all downstream events. Wu et al. have shown that ELKS siRNA prevents the activation of NF-κB by DSB too (Fig. 1) [89].

Historically, the downstream events of IKK activation were the first identified and found to be identical in nature to the cytokine-elicited ones but with fundamentally different kinetics and amplitude [30,31]. The phosphorylation of IκBα on S32 and S36 is easily detected within minutes after cytokine addition, whereas it takes an hour to be faintly detected after DSB formation as only a fraction of IκBα is phosphorylated. Similarly, the subsequent ubiquitination and degradation by the 26S proteasome are identical in nature but not in intensity. The dimer p50/p65 is the main NF-κB dimer translocating into the nucleus after DSB but its induction is slow and stable for about 4 h contrarily to what is observed with cytokines that trigger a faster but more transient activation. These differences, both in kinetic and signal amplitude, reflect the activation of the IKK complex itself. After cytokine addition, the IKK complex is rapidly recruited to the membrane receptor and activated; whereas, after DSB formation the posttranslational modifications of NEMO (sumoylation/phosphorylation/ubiquitination) are slow and rate-limiting. The difference in amplitude could result from the small amount of modified NEMO leaving the nucleus leading to a limited quantity of activated IKK complex relaying the DSB signalling cascade.

7. Functionality of DSB-initiated NF-κB

Once in the nucleus p50/p65 dimer affects the transcription of its numerous target genes. Early experiments, performed with cells expressing a non-degradable form of IκBα, preventing the nuclear shuttling of p50/p65 and thereby silencing all its transcriptional effects, have established that NF-κB activation is favourable to cell survival after CPT, VP16 and IR treatments [30,31,91,92]. Following treatment with these therapeutic agents, p65 is a transcriptional activator of many antiapoptotic genes and thus NF-κB is acting as prosurvival factor. The role of NF-κB as a major prosurvival actor challenging these therapies as been observed with tumours of different origins and is largely documented [34,93,94].

Dox can induce two NF-κB antagonist responses depending on cell types. In many tumour cells, p50/p65 induced by this drug is a gene activator and favours cell survival, as observed after CPT, VP16 or IR treatment [95–98]. In other cells, such as HEK 293, MDA-MB-231 (breast cancer cells) and U-2Os (osteosarcoma), Dox induces p50/p65 translocation into the nucleus where it binds normally to κB targets. In these cells however, p65 can act as a gene repressor instead of a gene activator. The repression is only observed with a subset of the κB responsive elements, notably antiapoptotic genes such as Bcl-xL and XIAP. The downregulation of these genes favours

apoptosis [8,99–101]. The repression is p65-dependent and results of the lack of post-translational modifications (phosphorylation and acetylation) of the NF-κB subunit. In addition, Dox-induced p65 is also able to exert a dominant negative effect over TNFα-induced p65 and thus completely modify the transcriptional response [99,101]. Other DNA topoisomerase II inhibitors share the same property. In a comparative study of six different topoisomerase II inhibitors, Campbell et al. have demonstrated that the transcriptional repression, observed with these inhibitors, correlates with their ability to intercalate DNA [22].

It is thus clear that the nature of the initial genotoxic signal(s) and the cell type dictate the function of NF-κB, which can either be pro- or antiapoptotic. This has important implications for therapeutic strategies aiming to inhibit the NF-κB cascade in order to limit the apparition of chemoresistance.

8. NF-κB inhibitors and sensitization to anticancer therapies

Following IR, CPT or VP16 treatment, NF-κB has a prosurvival function. Thus, its inhibition displaces the life or death balance of the cancerous cells towards apoptosis, increasing the efficiency of anticancer treatment and preventing tumour from becoming resistant. A large variety of inhibitors has been identified and are presently tested in models or evaluated in clinical trials. These inhibitors target different steps of the signalling pathway such as the proteasome or the IKK complex and were recently reviewed [34,102]. Following Dox or other intercalating topoisomerase II poisons treatment, NF-κB has proapoptotic functions in some tumour models and antiapoptotic function in other ones. Therefore, inhibitors designed to suppress this response may be deleterious instead of being beneficial. Finally, when studying the potential benefice of NF-κB inhibitors one has to take into account that NF-κB also mediates inflammation. After IR, the inflammation mediated by TNFα participates to the killing of the tumour cells. Thus, NF-κB inhibition will on one hand decrease the prosurvival function of the transcription factor but on the other hand decrease the lethal effect mediated by TNFα [34]. Consequently, the benefice of the inhibition may be attenuated. Additional studies to determine the potential of the inhibitors in cancer therapy are still needed to clarify this complex situation.

9. DSB trigger both pro- and antiapoptotic transcriptional responses

As stated earlier, the cellular responses to DSB are numerous and the cell fate depends on a delicate balance between pro- and antiapoptotic signals.

Upon DSB formation, PIDD and ATM are activated by a yet unidentified stress-related signal and DSB, respectively. Both signals converge to a common pathway to activate NF-κB (with prosurvival functions after IR, CPT and VP16 treatments) (Fig. 4). However, PIDD and ATM are double agents also controlling proapoptotic pathways. ATM simultaneously activates its proapoptotic and prosurvival pathways, whereas PIDD acts more like a switch, as the binding of RAIDD and RIP to PIDD seems to be mutually exclusive [72]. PIDD complexes are sequentially formed, the PIDD/RIP/NEMO prosurvival complex precedes the formation of PIDD/RAIDD/caspase 2 proapoptotic complex [72]. As p53 induces PIDD, the proapoptotic pathways initiated by ATM and PIDD are not completely independent [71].

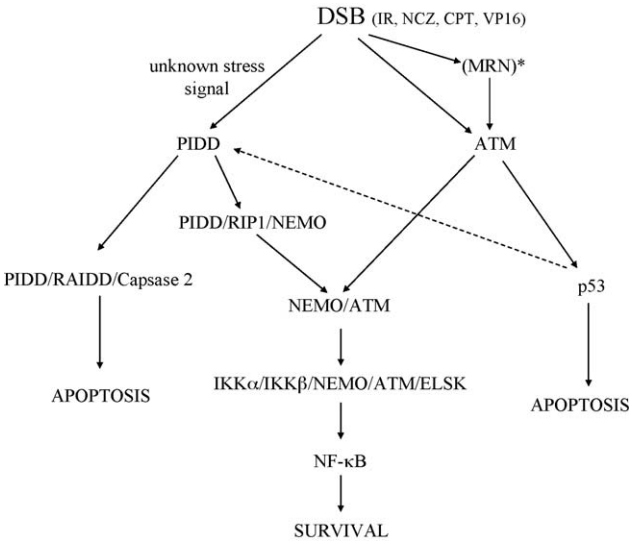

Fig. 4 – Both PIDD and ATM initiate antagonist pro- and antiapoptotic responses that influence the fate of the cells after DSB. In response to DSB, PIDD as well as ATM are capable of initiating cascades leading to pro- or antiapoptotic signals. The proapoptotic cascades have different intermediates, whereas the prosurvival cascade controlled by NF-κB is common. *MRN is only necessary for ATM activation when low DNA damage levels are generated. Adapted from ref. [72].

In 2005, Elkon and co-workers have compared the transcriptional network activated by NCS using microarrays and siRNA designed to knockdown ATM, p53 or p65. The effect of the suppression of ATM and its two downstream transcriptional factors was analyzed. They observed that NF-κB and p53 mediate most of the damage-induced gene activation controlled by ATM and that these two pathways are disjoints [103]. Thus, in normal cells where both pathways are present, DSB simultaneously activate pro- and antiapoptotic signals; cell survival or death depending on the amount and severity of lesions and on the repair capacities. In cancerous cells, however, the situation is different as p53 is often mutated. This leaves the cell with an unbalanced prosurvival signal that will favour the apparition of mutations and the development of resistance. Ideally, if one wants to decrease this phenomenon and potentiate the treatment, two conditions should be fulfilled: (i) the inactivation of NF-κB and (ii) the restoration of p53 proapoptotic pathway [103,104].

10. Conclusions

Major actors of the DSB-initiated cascade leading to NF-κB activation are now identified and positioned. However, several

questions remain to be addressed. What is the precise nature of the PIDD activating signal? What are the E2 and E3 ligases and hydrolyses responsible for NEMO sumoylation and desumoylation, respectively? How can p65 switch from gene activator to gene repressor when the DSB-generating compounds are also intercalating agents?

In view of our present knowledge of the DSB-signalling cascade leading to NF-κB activation, selective targets (such as the sumoylation of NEMO) could be used to enhance anticancer therapy. The inhibition of NF-κB will be beneficial for most genotoxic treatments inducing DSB (IR, CPT, VP16) but could be detrimental for Dox or other intercalating DNA topoisomerase II poisons depending on the cell type.

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