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Differential signaling circuits in regulation of ultraviolet C light-induced early- and late-phase activation of NF- κ B

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Abstract

Ultraviolet C light (UVC) induces NF- κ B activation via a complex network. In the early phase (4–12 h) of irradiation, NF- κ B activation is accompanied with I κ B α reduction via a translation inhibition pathway. In the late phase of UV-induced NF- κ B activation (16–24 h), the I κ B α depletion is a combined result of regulation at both transcriptional and translational levels. However, the NF- κ B activation appears to be independent of the level of I κ B α . In this review, we will discuss the multiple signaling circuits that regulate NF- κ B activation during the early and late phases of UVC-irradiation.

Introduction

The nuclear factor-kappa B (NF- κ B) plays important roles in the regulation of the expressions of genes that are mostly related to the immune and inflammatory response, along with genes determining developmental processes, cellular growth, and apoptosis (1–4). The canonical NF- κ B activation pathway is that upon stimulation, such as with tumor necrosis factor α (TNF α), an inhibitor of NF- κ B (I κ B) kinase (IKK) is activated and phosphorylates I κ B α at N-terminal serines (Ser32 and Ser36) (5,6). The phosphorylation leads to the dissociation of I κ B α from NF- κ B. While the phosphorylated I κ B is ubiquitin targeted and rapidly degraded through the polyubiquitin-dependent proteasomal pathway, the freed NF- κ B translocates into the nucleus with its exposed nuclear localization signal peptide (NLS) and activates its target genes (7–13). UV is known to activate NF- κ B (14,15), though the detailed mechanism is still under elucidation. In this review, only UVC-induced NF- κ B signaling circuits will be focused on since UVC is the strongest (14) and best studied for induction of NF- κ B activation among the three wavelengths, UVA, B and C. UVC-induced activation of NF- κ B does not always follow the canonical pathway (15,16). Compared to other stimuli such as TNF α , UVC activates NF- κ B in a delayed and prolonged manner with differential regulatory mechanisms for early- (within 12 h) or late-phase (within 16–24 h) post-UVC (15–17).

Early-phase activation of NF- κ B after UVC

IKK activation or N-terminal serine phosphorylation of I κ B α is not increased during the early-phase activation of NF- κ B after UVC-irradiation (16). However, while IKK activation is not detected above the basal level post-UVC, IKK activity is required and the IKK targeted serine sites on I κ B α are critical for UVC-induced NF- κ B activation (17). Several mechanisms were proposed to elucidate the early-phase activation of NF- κ B after UVC-irradiation.

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The role of translational inhibition in regulation of UVC-induced activation of NF- κ B

UVC does not accelerate the degradation of I κ B α in the early-phase of irradiation because the half-life of I κ B α is not reduced during the period (unpublished data). Instead, I κ B α synthesis is reduced in accompany with the inhibition of nascent protein synthesis post-irradiation. Since the half-life of I κ B is only 140 minutes for complexed endogenous I κ B and 40 minutes for free, overexpressed I κ B (16), a reduced expression of I κ B α leads to a reduction in the net amount of I κ B and sequentially NF- κ B activation (18,19). The down regulation of I κ B α expression is controlled at the translational level via the phosphorylation of alpha subunit of the eukaryotic initiation factor 2 (eIF2 α), which plays a critical role in the regulation of protein synthesis. The phosphorylation of eIF2 α at the Ser51 inhibits translational initiation (20,21) and activates NF- κ B (22). Four protein kinases are known to phosphorylate Ser51 in eIF2 α in response to different stress stimuli. All four eIF2 α kinases (EIF2AK) have been shown to be directly or indirectly involved in NF- κ B activation (23). Among them, the dsRNA-dependent protein kinase-like endoplasmic reticulum (ER) kinase (PERK, EIF2AK3) and the general control nonderepressible protein kinase 2 (GCN2, EIF2AK4) are activated and mediate NF- κ B activation after UVC-irradiation (18,19,24,25).

UVC-induced eIF2 α phosphorylation is a prolonged process. Due to the delayed activation of eIF2 α kinases, UVC was believed not to be an inducer of eIF2 α phosphorylation (26). The eIF2 α phosphorylation was first detected at 4 h post-UVC and PERK was identified as the mediator for UVC-induced phosphorylation of eIF2 α (24). Soon after, GCN2 was also identified as a kinase that phosphorylates eIF2 α upon UVC-irradiation (25). Both PERK and GCN2 regulate the early phase activation of NF- κ B (18,19). The UVC-induced eIF2 α phosphorylation and NF- κ B activation were significantly inhibited in PERK or GCN2 knockout mouse embryonic fibroblast (MEF) cell lines (18,19). Analysis of NF- κ B activation in a MEF cell line containing an eIF2 α mutant in which Ser51 was mutated to alanine (MEF^{A/A}) showed similar inhibition of NF- κ B (18,19). Based on the fact that IKK activity is not induced but is required for UVC-induced I κ B α reduction; PERK and GCN2 mediate UVC-induced eIF2 α phosphorylation and translational inhibition of I κ B synthesis, a model for UVC-induced early phase activation of NF- κ B was proposed. UVC activates PERK and GCN2, which phosphorylate eIF2 α and inhibit new I κ B α synthesis. The existing I κ B amount is reduced upon natural degradation through the polyubiquitine pathway and finally NF- κ B is activated due to the lack of I κ B (Fig. 1).

The contribution of IKK in reduction of I κ B α upon UVC-irradiation

IKK is a 700 kDa protein complex consisting of two catalytic subunits (IKK α and IKK β) and a regulatory subunit NEMO (NF- κ B essential modulator or IKK γ) (11,17,27). Activation of the catalytic subunits takes place by intra- and inter-molecular phosphorylation followed by releasing of their kinase domains, which phosphorylate I κ B α at Ser32/36 and promote its degradation (5,6). The reduction of I κ B in the early phase of UVC-irradiation does not apparently involve a detectable IKK activation and I κ B α phosphorylation at Ser32/36 (15,16). However, the N-terminus of I κ B α is required for I κ B α reduction after UVC since a deletion mutant of I κ B α lacking 36 amino acids at N-terminal is stable upon UVC-irradiation (16). Interestingly, the UVC-induced NF- κ B activation is abolished in a cell line that is stably transfected with I κ B α (S32/36A) mutant, which has both S32/36 replaced with Ala, while transient transfected I κ B α (S32/36A) mutant is not resistant to UVC-induced reduction. These results suggest that while the UVC-induced reduction of excess amount of free I κ B α is independent of IKK activity, the degradation of NF- κ B-bound I κ B α requires active IKK (28).

Further study indicates that UVC-irradiation slightly increases phosphorylation of Ser32/36 on overexpressed I κ B α , which can only be detected after capturing by an overexpressed F-

box deletion mutant of ubiquitin ligase β -TrCP as a specific phosphoprotein substrate trap after immunoprecipitation. In addition, deletion of 25 amino acids at C-terminal of NEMO, which contains a putative Cys₂HisCys zinc finger motif (ZF), abolishes the UVC-induced activation of NF- κ B (17). Based on these results, it is proposed that while an increased activation of IKK is not detected after UVC-irradiation, IKK activity still plays a critical role in regulating I κ B α degradation via phosphorylation of Ser32/36 (Fig. 1).

The roles of CK2 (Casein Kinase 2) in removal I κ B α upon UVC-irradiation

In addition to the translational inhibition of I κ B α synthesis and IKK-mediated I κ B α degradation, CK2 also plays roles in regulating I κ B degradation and NF- κ B activation upon UVC-irradiation. In response to UVC, CK2 is activated via p38 MAPK pathway and phosphorylates I κ B α at a cluster of C-terminal sites, which leads to I κ B α degradation and NF- κ B activation (29). Knockdown of CK2 using siRNA eliminates UV-induced I κ B α degradation (29) while expression of an I κ B α mutant with 6 CK2 target sites replaced with Ala attenuates UV-induced NF- κ B activity (28). UVC-activated CK2 also phosphorylates β -arrestin, which interacts with I κ B α and stabilizes the I κ B α •NF- κ B complex (30). The phosphorylation of β -arrestin reduces its binding affinity to I κ B α and leads to the dissociation of I κ B α from the I κ B α •NF- κ B complex.

Regulation of NF- κ B activation

The UVC-induced early phase activation of NF- κ B is independent of nuclear signal generated by DNA damage, but dependent on the activation of Src-Ras-Raf signaling cascade (16,31). Overexpression of dominant negative mutant of v-src, Haras, or raf-1 inhibits UVC-induced early activation of NF- κ B. Since both Src and Ha-Ras resident on plasma membrane, it is suggested that the UVC-induced signaling cascade for activating NF- κ B is initiated on or near the plasma membrane (31). In the meantime, while IKK is important in regulation of I κ B level as discussed above, NEMO, the regulatory subunit of IKK, appears to play an additional role in recruiting cofactor(s) that are critical for UVC-induced NF- κ B activation (17). The interaction between NEMO and the catalytic subunits is required for the activation of NF- κ B induced by UVC-irradiation. Furthermore, overexpression of NEMO restores and robustly increases the inducibility of NF- κ B after UVC-irradiation in a NEMO deficient cell line, which suggests that expression level of NEMO may be the rate-limiting step of the UVC-induced NF- κ B signaling pathway.

In spite of the kinase-binding domain which interacts with IKK α and IKK β , NEMO also contains two coiled-coil domains, a leucine zipper motif (LZ) and a putative zinc finger domain (ZF) at the C-terminal. NEMO is able to be self-multimerized, either in a trimerization form or a tetramerization form through the LZ and C-terminal coiled-coil domain (32-34). The ZF itself is not involved in the multimerization since deleting the ZF does not significantly affect the multimerization of NEMO (35). However, the extra ZF that added on to NEMO dimer via multimerization might assist the LZ to recruit upstream factor(s) to promote NF- κ B activation (32). The vital role of the NEMO ZF in recruiting activator(s) from upstream signaling pathways is also applicable to UVC-induced NF- κ B activation since expression of ZF mutant with amino acid substitution abolishes the inducibility of NF- κ B upon UVC-irradiation (17). Since the ZF is able to interact with a variety of different molecules (36), it is not clear though whether the ZF of NEMO recruits upstream cofactor(s) to activate IKK or to directly activate NF- κ B (Fig. 1)

Late Phase activation of NF- κ B after UVC

The regulation of the late phase activation of NF- κ B after UVC exposure is more complex than the original thought (15,16). During the period post-UVC, IKK is activated, which

phosphorylates the N-terminal Ser32 and Ser36 of I κ B α and promotes I κ B α degradation (16). Meanwhile, UVC-induced eIF2 α phosphorylation down-regulates I κ B expression at both transcriptional and translational levels (37). However, the reduction of I κ B may not be a direct cause of NF- κ B activation (37). Furthermore, neither ubiquitination nor proteasomal degradation has detectable contribution to late-phase UV-induced I κ B α depletion (37). Some contradictory theories and potential signaling circuits in regulation of NF- κ B activation in the late phase of UVC are discussed below.

Regulation of I κ B reduction

The late phase activation of NF- κ B was suggested to be dependent on a DNA-damage-induced and IKK-mediated I κ B degradation pathway (15,16). In the late phase of UVC exposure, DNA-damage and the production of IL-1 α and autocrine/paracrine induced by DNA-damage were crucial for NF- κ B activation. Moreover, unlike early phase, late phase required both IKK and phosphorylatable I κ B at Ser32/36 for NF- κ B activation (16). The level of I κ B is also regulated by eIF2 α . Our previous data showed that at 24 hr after UVC exposure, the mRNA level remained unchanged in MEF^{S/S} cells but significantly increased in MEF^{A/A} cells, as well as decreasing of I κ B protein level in MEF^{S/S} cells and the intact protein level in MEF^{A/A} cells. These results mean that at late onset of NF- κ B activation caused by UVC irradiation, the phosphorylation of eIF2 α inhibited both the transcriptional and translational level of I κ B α and thus regulated NF- κ B activation (Fig. 2).

The involvement of IKK in regulation of I κ B is challenged by the results showing that a membrane-permeable proteasome inhibitor MG132 and an I κ B α ubiquitin ligase inhibitor Ro106-9920 failed to influence I κ B α levels in MEF cells at 24 hours post-UVC (37). In addition, the phosphorylation of Ser536 of NF- κ B (p65), which is a direct target of activated IKK (38,39), is not increased at the late phase of the irradiation (37). These results suggest that there might be an IKK-dependent or -independent pathway existing in different cell lines that regulates I κ B level (Fig. 2).

I κ B-independent activation of NF- κ B

While I κ B is reduced in the late phase of the irradiation, NF- κ B activation does not appear to be dependent on the reduction. NF- κ B can be activated in the presence of normal level of I κ B in a non-phosphorylatable eIF2 α knock-in mouse fibroblast cell line during the late phase of UVC-irradiation (18,37). These results indicate that I κ B reduction in the late phase of the irradiation might not be a direct cause of NF- κ B activation and UVC induces the DNA-binding of NF- κ B (p65) (18). Further analysis on the critical phosphorylation sites of NF- κ B (p65) shows that an increased phosphorylation of Ser276 of NF- κ B (p65), but not Ser536, is correlated to the activation of the protein. Two kinases, mitogen and stress activated protein kinases (MSK) and protein kinase A (PKA) phosphorylate the Ser276 of NF- κ B (p65) in the nucleus (40) or in the cytosol (41-43) respectively. Since the phosphorylation levels of Ser276 of NF- κ B (p65) is only increased in the nucleus, but not in cytosol, MSK signaling cascade is more likely to be the mediator of UVC-induced late-phase activation of NF- κ B (Fig. 2).

Translocation and DNA binding of NF- κ B

During the late-phase of UVC-irradiation, NF- κ B is activated with increased DNA-binding activity (18) and p65(Ser276) phosphorylation (37). The phosphorylation of p65(S276) is increased even in the presence of normal level of I κ B in the nucleus, but is not detected in the cytosol. Since UVC is able to induce NF- κ B translocation without activation in cells that overexpress a dominant negative PERK (18), it is likely that UVC is able to induce an I κ B-independent release and translocation of NF- κ B into nucleus, where it is phosphorylated and activated by MSK (Fig. 2).

In addition to direct regulation of the activation of transcription factors, such as NF- κ B, MSK is also able to remodel chromatin, which further regulates transcriptional activity. MSK has two isoforms – MSK1 and MSK2. Both isoforms of MSK are capable of phosphorylating histone 3 (H3) at Ser10 and Ser28 (44-46). However MSK2 has been observed to play a more crucial role in H3 modification (45). Phosphorylation of either of these sites on H3 results in a conformational change, as well as a change in charge on the molecule; both of which play important roles in chromatin binding (44,46). In this way, UVC-induced activation of MSK might play dual roles in regulation of the transcriptional activity of NF- κ B via direct phosphorylation of NF- κ B as well as modification of the availability of genes for transcription (Fig. 2).

Summary

UVC-induced activation of NF- κ B has two distinct phases. In the early phase following irradiation, I κ B α is reduced due to the combined effects of translational inhibition and IKK/CK2 activities. Meanwhile NEMO also recruits cofactor(s) that further promotes activation of the freed NF- κ B in the cytosol. In the late phase following irradiation, NF- κ B is released from I κ B with an unknown mechanism and translocates into the nucleus, where it is phosphorylated and activated by MSK. In the meantime, MSK phosphorylates chromatin, which further regulates the transcription of genes that are under control of NF- κ B.

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Biographies

Shiyong Wu is currently a Principal Investigator in Edison Biotechnology Institute and an Associate Professor in the Department of Chemistry and Biochemistry at Ohio University, Athens, OH. With his interest in the mechanisms underlying the regulation of protein expression and function by free radicals and membrane lipids, his lab is dynamically and systematically analyzing the coordinative effect of small molecules, such as reactive oxygen and nitrogen species (ROS, RNS) and membrane lipids, in regulation of biochemical and biophysical properties of cells after UV-irradiation.

Lingying Tong received her Bachelor of Science Degree from the Department of Biochemistry, the Chinese University of Hong Kong. She joined Dr. Wu's laboratory as a graduate student in the Molecular and Cellular Biology program/Department of Chemistry and Biochemistry at Ohio University in 2009. One of her current research projects is to identify the role of NOS and oxidative stress in UV-induced NF- κ B activity.

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Figure 1.
Signaling circuit that regulates UVC-induced early-phase activation of NF- κ B.

Figure 2.
Signaling circuit that regulates UVC-induced late-phase activation of NF-κB.