Chapter 8

Maintenance of Genome Stability by Ubiquitination of DNA Repair Proteins in Mammalian Development and Disease

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Additional information is available at the end of the chapter

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Abstract

To maintain genome DNA, DNA repair machinery has been developed in cellular life cycle. Multiple DNA repair pathways such as base excision repair, nucleotide excision repair, DNA cross link damage repair, DNA single strand break repair and DNA double strand break repair including nonhomologous end joining and homologous recombination are regulated by protein signal cascade. Because of limited gene number, protein posttranslational modification signal has advantage to control cell dynamics during development and senescence. This chapter focuses on how DNA repair proteins molecular modification including phosphorylation and ubiquitination contribute to genome stability pathway during mammalian development and disease.

Keywords: DNA repair, BRCA1, FA pathway, NBS1, mammalian development, inherited disease

1. Introduction

Genome DNA is damaged by several environmental factors such as ionizing radiation (IR), ultraviolet (UV), environmental mutagen and metabolic products including reaction oxygen species (ROS). It is well known that IR induces base damage, DNA cross link and DNA strand breaks defined as, single strand breaks (SSB) and double strand breaks (DSB) [1] For instance, 1Gy gamma-ray irradiation induces 1000 SSBs and 40 DSBs per cells [2] Although base damage causes genome DNA mutation leading to cancer, DSB is a catastrophic damage that leads to severe chromosome breakage and cell death. To prevent this,

in mammalian cells, there are two major DSB repair pathway: nonhomologous end joining (NHEJ) repair [3] and homologous recombination (HR) repair [4] (**Figure 1**). NHEJ repair joint DNA damage ends directly and act as dominant repair pathway through cell cycle. HR repair is a precise pathway to repair completely with sister chromatid during S-G2 phase.

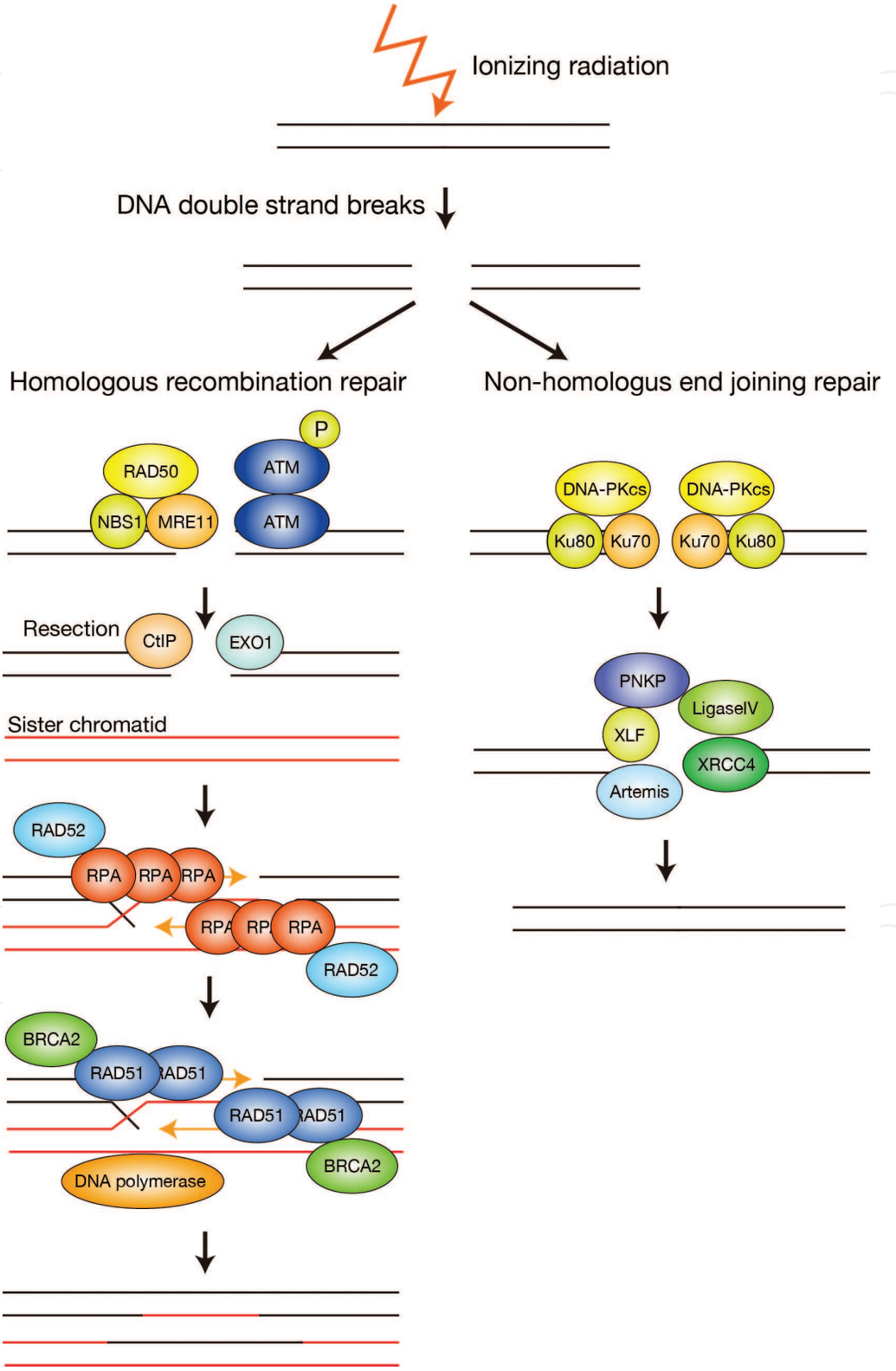

Figure 1. Schematic model of HR repair and NHEJ repair.

Both pathways cooperate to maintain genome DNA stability. Defect or depletion of related proteins of DNA repair result in hypersensitivity to the IR, severe developmental failure especially central nervous system and predisposition to the cancer. Recently, it is reported that abnormal expression of DSB repair proteins causes neurodegeneration, and small head phenotype called microcephaly. Neural stem cells and progenitors actively proliferate and produce ROS from mitochondria respiration [5, 6], which attack genome DNA. Decreasing of DNA repair activity is critical for cell survival. Furthermore, atomic bomb survivors show microcephaly when they are exposed in the womb [7]. DNA damage and centrosome amplification are critical for mammalian embryonic brain development [8, 9]. To regulate DNA repair machinery, several protein posttranslational modification systems such as phosphorylation, ubiquitination, SUMOylation, and NEDDylation are involved [10]. In this chapter, to identify how DNA repair signaling pathways are involved in mammalian development and disease, ubiquitination system in DNA repair machinery are focused on and discussed.

1.1. DSB NHEJ repair pathway

DNA-PKcs, Ku70, Ku80 are main components of NHEJ pathway and recruited to DNA damaged ends immediately when DSB occurred. Binding of Ku70/Ku80 to the DNA ends is important for protection from resection and recognition as telomere ends [11, 12]. DNA-PKcs act as a signal inducer by phosphorylation of several substrates [13]. DNA-PKcs also phosphorylates itself to activate this pathway. Subsequently, XRCC4 is recruited to damage sites as scaffold and then related proteins such as XLF, PAXX and Artemis are accumulated [14]. Artemis has endonuclease activity to resect DNA ends to facilitate DNA ligation. Polynucleotide kinase phosphatase (PNKP) is also recruited to DNA ends to remove 3′-P groups or add 5′-P residues for ligation [15, 16]. Finally, DNA ligase IV joint DNA ends. Because of broken ends by physical damage and resection by Artemis, NHEJ pathway sometimes generates DNA mutation and deletion. NHEJ pathway is not only important for DNA repair pathway, but also immune-systems, V(D)J recombination [17, 18]. Human patients with deficiency of Artemis, XLF, Ligase IV show severe combined immunodeficiency (SCID) and microcephaly phenotype. To investigate the role of DNA repair machinery in central nervous system, brain-specific conditional knockout mice of Ligase IV and PNKP were generated [19]. These mice show severe DNA damage and apoptosis in the cerebral cortex during embryonic development. This suggests that NHEJ repair machinery is important for genome maintenance of neural stem cells and progenitors during brain development.

1.2. DSB HR repair pathway

HR repair is a precise repair pathway that requires sister chromatid as DNA template. Ataxia telangiectasia mutated (ATM) and MRE11/RAD50/NBS1 (MRN complex) are accumulated to DNA damage sites as DNA damage sensor and ATM phosphorylates ATM itself (auto-phosphorylation) to activate HR signal [20, 21]. ATM is master regulator of DNA damage response signaling and has many substrates such as p53, SMC1, NBS1, MDC1, 53BP1 and CHK2. ATM regulates G1/S, S and G2/S cell cycle checkpoint. Ataxia telangiectasia (AT) patients show hypersensitivity to the IR, immunodeficiency, predisposition

to malignancy and progressive cerebellar neurodegeneration. *MRE11* is responsible gene for AT like disorder (ATLD). ATLD patients show similar phenotype with AT. MRE11 has nuclease activity to generate 3' ssDNA tails. Detail of NBS1 discussed below. After ATM activation, signal mediator such as BRCA1 and 53BP1 are accumulated depending on ubiquitination of histone protein by RNF8. RNF8 and RNF168 are key E3 ubiquitin ligase in HR pathway [22, 23]. Poly-ubiquitination of histone H1 by RNF8 is important for RNF168 recruitment to the DNA damage sites. Chromatin remodeling associates with DNA damage response to facilitate DNA repair. Histone protein modification is trigger of this process. NBS1 and ATM interact with E3 ubiquitin ligase RNF20 which mono-ubiquitinates H2B after DNA damage [24, 25]. RNF20 dependent H2B mono-ubiquitination is important process of DNA repair, because depletion of RNF20 by siRNA reduces accumulation of RAD51 and BRCA1 to the DNA damage sites. DNA exonucleases CtIP and Exo1 are recruited to damage sites to resect DNA ends [26]. Then, Replication protein A (RPA) and RAD52 binds to single strand DNA ends to protect DNA and replace with RAD51 and BRCA2 to promote DNA recombination [27, 28]. Simultaneously, cell cycle checkpoint proteins, p53, CHK1 and CHK2 activate to give repair time. HR factors are not only involved in DNA repair, but also meiosis.

Both DNA repair pathway are strictly regulated, and several E3 ubiquitination ligases are involved in these [29]. Defect of DNA repair machinery leads to chromosome aneuploidy and several diseases such as neurodegeneration, inflammation and cancer [30].

1.3. NHEJ and HR proteins and centrosomes

As mentioned earlier, NHEJ and HR proteins are important for DNA repair to maintain genome stability. In fact, defect of NHEJ and HR proteins leads to severe inherited disease such as immunodeficiency, neurodegeneration, developmental defect, predisposition to the malignancy. Recent reports uncover that NHEJ proteins and HR proteins localize centrosomes. Centrosome is an organelle consists of two centrioles surrounded by pericentriolar material (PCM) [31, 32]. γ-tubulin ring complex (γ-TuRC) attaches PCM to form microtubule extension. Centrosome plays pivotal role for the proper cell division [33]. Mammalian cells usually have one or two centrosomes depending on cell cycle. Ionizing radiation (IR) or some genotoxic reagents trigger centrosome amplification, which cause multipolar cell division and chromosome aneuploidy [8, 34–37]. Centrosome duplication is basically regulated cell cycle machinery. NHEJ factors such as DNA-PKcs localize centrosomes [38]. We found that DNA-Pkcs or Ku70 deficient murine embryonic fibroblast (MEF) cells show slight centrosome amplification compared with complementary cells. Meanwhile, HR factors such as ATM, NBS1, BRCA1, BRCA2 and Rad51 localize centrosome and depletion of these factors show significant centrosome amplification [39]. The role of NBS1 and BRCA1 in centrosome maintenance is discussed in the following section. ATM phosphorylates centrosome protein CEP63 to regulate spindle assembly after DNA damage [40]. Inhibition of ATM in RAD51 deficient cells shows centrosome amplification which means that ATM and RAD51 interaction is important for centrosome proper duplication [41]. BRCA2 interacts with NPM to form BRCA2-NPM complex to maintain centrosome duplication and cell division [42]. CHK1 is one of key regulator for cell

cycle checkpoint to activate G2/M checkpoint. Depletion of CHK1 expression leads to centrosome amplification [41, 43]. After DNA damage, ATM- and Rad3-related (ATR) phosphorylates CHK1 to move to the centrosome from nucleus. ATR-dependent CHK1 translocation is important for centrosome duplication after DNA damage [44, 45]. Recent reports unveiled importance and molecular mechanism of HR factors in centrosome maintenance. However, function and physical means of NHEJ factors in centrosome still remain unclear.

2. Ubiquitination of DNA repair proteins and development

2.1. E3 ubiquitin ligase BRCA1

Breast and ovarian cancer gene, BRCA1 have multiple function in cell metabolism, including DNA repair, chromatin remodeling, microtubule maintenance and centrosome duplication. About 10% of women patients with breast cancer have inherited mutations in BRCA1 or BRCA2. BRCA1 forms heterodimer with BRCA1-associated RING domain (BARD1) to act as E3 ubiquitin ligase, which has several substrates including H2A, H2AX, RNA pol III, THIIE, NPM1, CtIP, ER-α and claspin [46–51] (**Figure 2**). Since BRCA1 can mono-ubiquitinates H2A and H2AX in vivo, it is believed that BRCA1 is required for chromatin remodeling after DNA damage [52, 53].

2.2. BRCA1 and centrosomes

BRCA1-BARD complex mono-ubiquitinates γ-tubulin at Lysine 48 and Lysine 344, which is the main component of centrosome [54–57]. Previously, we reported that Nijmegen breakage syndrome (*NBS*) gene and *ATR* gene products, NBS1 and ATR are involved in BRCA1 dependent γ-tubulin mono-ubiquitination to regulate centrosome duplication [58, 59]. Deficient of BRCA1 leads to centrosome amplification. Furthermore, BRCA1 and NBS1 are required for suppression of low dose rate IR dependent centrosome amplification [60]. This result suggests that BRCA1 keep genome integrity through cell cycle. So far, it is not known de-ubiquitinating enzymes (DUBs) of γ-tubulin. CP110 is a centriolar protein that regulates centrosome duplication. The level of CP110 is regulated by ubiquitination and de-ubiquitination by ubiquitin ligase complex $SCF^{cyclinF}$ and DUB USP33, respectively [61]. Destabilized CP110 levels by ubiquitination status lead to centrosome amplification and genome instability. Thus, balance of ubiquitination status is important. To identify DUBs of BRCA1-dependent γ-tubulin ubiquitination will contribute therapeutic strategies.

2.3. Mouse model of BRCA1

Since BRCA1 is involved in multiple cellular functions, complete defect of that leads to embryonic lethality. To identify the role of BRCA1 in mammalian development, conditional knockout mice were generated. Deletion of BRCA1 in mammary gland result in a phenotype of human basal like breast cancer [62, 63]. Central nervous system (CNS) specific BRCA1 knockout using nestin promoter resulted in microcephaly [64, 65]. Apoptotic cells were increased in brain layer structure during embryonic stage in BRCA1 brain specific KO mice. As another possibility, since genetic background such as Plk4 overexpression or genotoxic stress such as IR induce

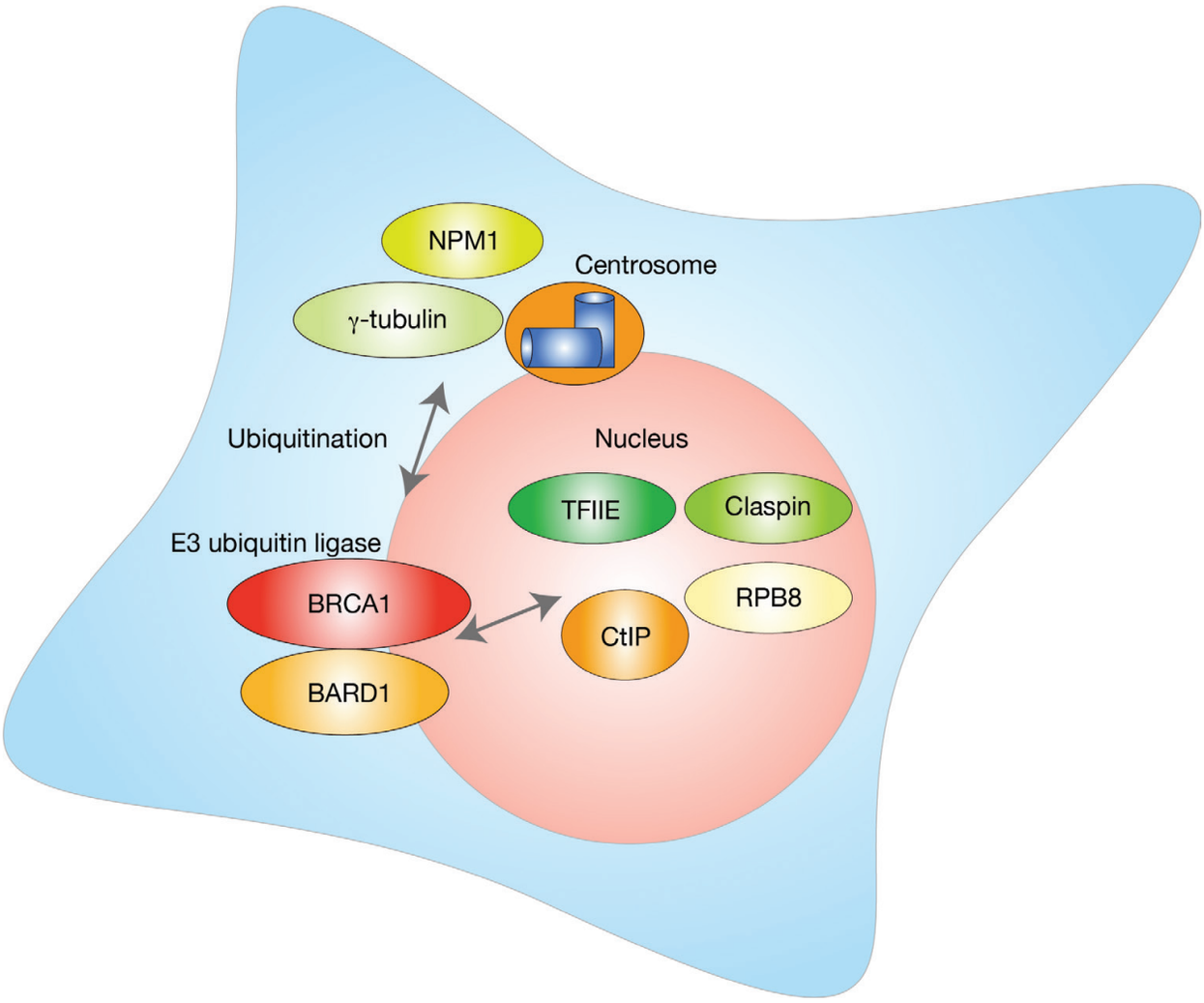

Figure 2. BRCA1 and its substrates.

centrosome amplification during CNS development result in microcephaly, centrosome amplification might be involved in microcephaly formation in BRCA1 deficient mouse brain [8, 66]. This result suggests that BRCA1 is important for genome maintenance in mammalian neural development.

2.4. Fanconi anemia (FA) pathway

Fanconi anemia (FA) is a hereditary disease clinically characterized as skeletal and visceral malformations, attrition of bone marrow stem cells [67, 68]. FA is firstly reported by Fanconi in 1927 and founded to be sensitive to DNA cross link damage by Sasaki et al. [69]. FA proteins pathway is important for inter cross link (ICL) DNA damage repair and HR repair [70, 71]. Currently, at least 21 FA proteins are reported. FANCD2 is a key player in FA pathway [72–75]. Mono-ubiquitination of FANCD2 at Lysine 561 by FA core complex is important event for activation of FA pathway. FA core complex consists by eight FA proteins (FANCA, B, C, E, F, G, L, M) and associated factors (FAAP100, FAAP24, FAAP20, MHF1 and MHF2). K561 mutated FANCD2 proteins cannot form DNA damage foci and localize to chromatin

suggest that mono-ubiquitination of FANCD2 is essential event for DNA repair. FANCD2 forms heterodimer with FANCI, which is phosphorylated by ATR-ATRIP complex. Mono-ubiquitination of FANCD2 is de-ubiquitinated by USP1 after completion of DNA repair [76, 77]. Knockout mice of USP1 show FA like phenotype. This suggests that regulation of mono-ubiquitination level of FANCD2 is critical for DNA repair pathway [76, 77].

2.5. Mouse model of FA proteins

Knockout mice of FA genes show decreasing of fertility and chromosome breaks [78–80]. *Fancg* knockout mice show germ cell defects and decreasing of fertility. *Fancg*−/− cells display high sensitivity to the IR and DNA crosslink inducer mitomycin C (MMC). Fancd2−/− mice show more severe phenotype characterized by perinatal lethality microphthalmia and hypogonadism. Fancd2−/− mice are also prone to developing epithelial cancers than Fanca−/−, Fancc−/− and Fancg−/− mice [78, 79, 81–84].

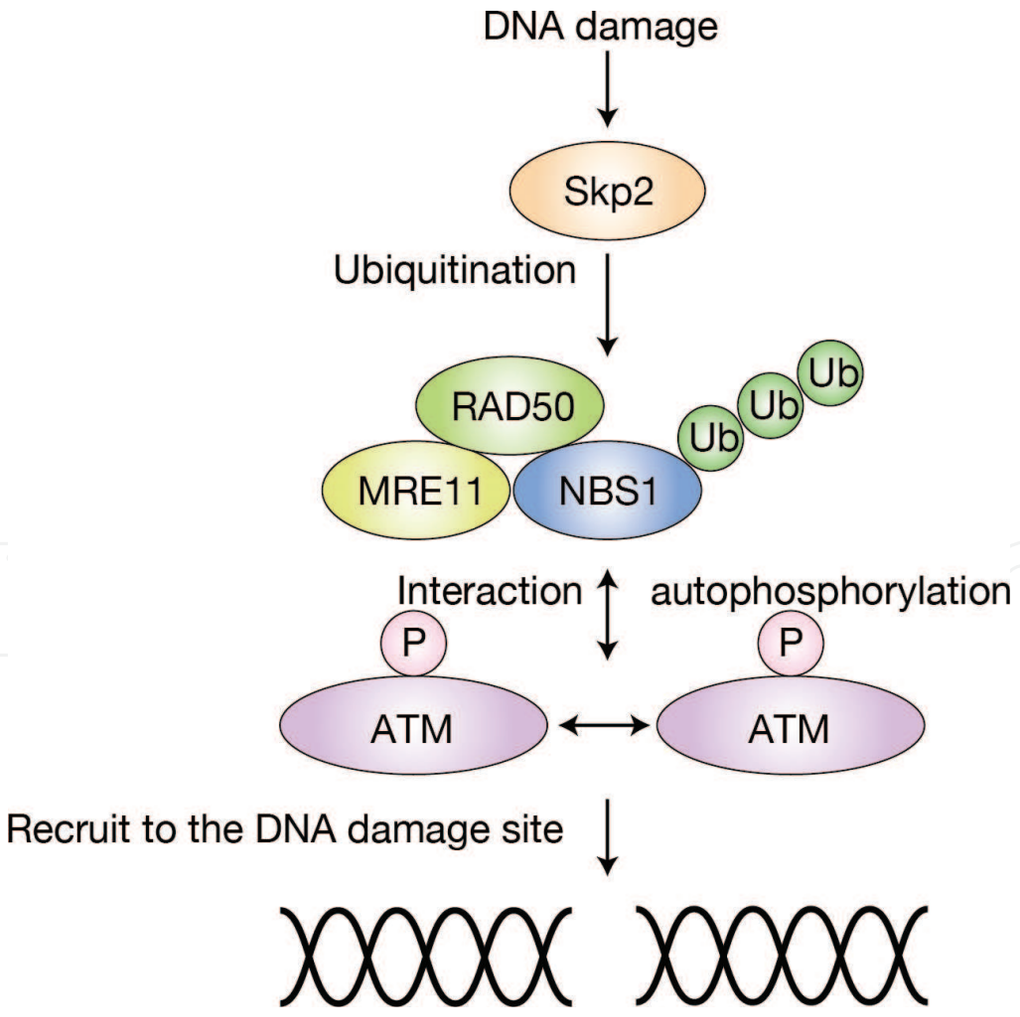

Figure 3. Ubiquitination of NBS1 by Skp2 is important for DNA repair pathway.

2.6. NBS1

Nijmegen breakage syndrome (NBS) is characterized by immunodeficiency, predisposition to the malignancy and IR hypersensitivity [85, 86]. Gene product NBS1 is 95 kDa protein and has several roles to maintain genome stability such as, HR repair, DNA replication initiation, cell cycle checkpoint, apoptosis, UV damage repair and centrosome duplication [58, 87–95]. NBS1 forms complex with MRE11 and RAD50 as MRN complex and act as DNA damage sensor and initiator [96]. Complete deletion of NBS1 proteins in mice leads to embryonic lethality. 70 kDa fragment of NBS1 protein expresses in NBS patient cells. NBS1 localizes to the nucleus and centrosomes. Depletion of NBS1 by siRNA in human osteosarcoma U2OS cells and murine embryonic fibroblast NIH3T3 cells show radio-sensitivity and centrosome amplification which suggest that NBS1 is required for DNA repair and centrosome duplication process. NBS1 is phosphorylated by ATM and ATR to activate G1/S checkpoint and G2/M checkpoint, respectively. NBS1 acts as DNA damage sensor and is important for ATM recruitment to the DNA damage sites. Ubiquitination of NBS1 by E3 ubiquitin ligase 3 Skp2 is required for interaction with ATM and activation [97] (**Figure 3**). Defect of Skp2 leads to decreasing of ATM foci formation at the DNA damage sites. Furthermore, NBS1 is involved in translesion DNA synthesis (TLS) [92]. After UV exposure, E3 ubiquitin ligase RAD18 recruited to the DNA damage sites and mono-ubiquitinates PCNA to initiate TLS. NBS1 controls RAD18 function because depletion of NBS1 results in decreasing of foci formation of pol eta and mono-ubiquitination of PCNA.

3. Concluding remarks

Genome DNA is attacked by several factors not only environmental stress but also metabolic stress to maintain cellular homeostasis. DNA repair and genome maintenance molecular mechanisms are strictly regulated by many enzymes. Since Goldstein and Ciechanover first reported about ubiquitin, the biological significance of this small peptide has been focused on several fields such as proteasome maintenance, translational signaling and DNA repair [98–100]. Ubiquitination of DNA repair factors are important for facilitates signaling cascade, because protein posttranslational modification is useful tool to diverse signaling pathway. DNA repair proteins defects cause several diseases such as immunodeficiency, neurodegeneration, growth defects and cancer progression. Furthermore, ubiquitination of DNA repair pathway is strong target for cancer therapy [101]. To understand of molecular pathway is necessary for clinical application.

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