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REVIEW

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The Nijmegen breakage syndrome gene and its role in genome stability

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Abstract NBS1 is the key regulator of the RAD50/MRE11/NBS1 (R/M/N) protein complex, a sensor and mediator for cellular DNA damage response. NBS1 potentiates the enzymatic activity of MRE11 and directs the R/M/N complex to sites of DNA damage, where it forms nuclear foci by interacting with phosphorylated H2AX. The R/M/N complex also activates the ATM kinase, which is a major kinase involved in the activation of DNA damage signal pathways. The ATM requires the R/M/N complex for its own activation following DNA damage, and for conformational change to develop a high affinity for target proteins. In addition, association of NBS1 with PML, the promyelocytic leukemia protein, is required to form nuclear bodies, which have various functions depending on their location and composition. These nuclear bodies function not only in response to DNA damage, but are also involved in telomere maintenance when they are located on telomeres. In this review, we describe the role of NBS1 in the maintenance of genetic stability through the activation of cell-cycle checkpoints, DNA repair, and protein relocation.

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Introduction

Dysfunction in the maintenance of genome stability can lead to malignant transformation, genetic mutations, and chromosome instability. Defective or mutated *NBS1* is the gene responsible for Nijmegen breakage syndrome (NBS), a disorder characterized by microcephaly, chromosomal instability, immunodeficiency, and a high incidence of malignancy. Cells from NBS patients were reported to display hypersensitivity to ionizing radiation and abnormal S-phase checkpoints (Shiloh 1997). These cellular phenotypes are identical to those seen in ataxia-telangiectasia (AT), which is caused by mutations in ATM, and in AT-like disease (ATLD), caused by mutations in MRE11. Cells derived from NBS patients also exhibit decreased homologous recombination (Tauchi et al. 2002), accelerated shortening of telomeres (Ranganathan et al. 2001), and disruption of the G1-, G2-, and intra-S-phase checkpoints after irradiation (Shiloh 1997; Ito et al. 1999; Buscemi et al. 2001; Girard et al. 2002; Lukas et al. 2003). These phenotypes indicate a critical role of NBS1 in maintaining genomic stability. In fact, NBS1 forms a complex with RAD50 and MRE11 (Paull and Gellert 1999; Lee et al. 2003), the so-called R/M/N complex, and regulates the catalytic activity and nuclear localization of the complex. Here, we summarize recent findings on the function of the NBS1 complex, which is involved in the DNA double strand end-processing pathway, and in protein nuclear bodies.

Structure and evolutionary conservation of the NBS1 protein

The *NBS1* gene contains 16 exons encompassing a genomic sequence of more than 48,979 bp on chromosome 8q21 (Tauchi 2000). The human NBS1 gene is transcribed as two mRNAs of 2.6 kb and 4.8 kb, which differ in the lengths of their 3′-untranslated regions. In contrast, mouse NBS1 is transcribed as a single mRNA of 2.8 kb (Wilda et al. 2000). There is a 50 bp pseudo-exon

from intron 2 of the *NBS1* allele, and it can cause alternative splicing of the gene (Takakuwa et al. 2004). The human NBS1 protein consists of 754 amino acids, and shows three functional regions: the N-terminus (amino acids 1–183), a central region (amino acids 278–343) and the C-terminus (amino acids 665–693) (D'Amours and Jackson 2002; Tauchi et al. 2001) (Fig. 1). A weak (29%) homology to the budding yeast (*Saccharomyces cerevisiae*) Xrs2 protein, which is related to radiation sensitivity and meiotic recombination, was first recognized at the N-terminal sequence. This region includes a fork head-associated (FHA) domain (amino acids 24–109) and a BRCA1 C-terminus (BRCT) domain (amino acids 114–183). FHA and BRCT domains are often found in eukaryotic nuclear proteins that are involved in cell-cycle checkpoints or DNA repair. The yeast BRCT domain has not been identified in budding yeast, whereas it is conserved in fission yeast (*Shizosaccharomyces pombe*) (Ueno et al. 2003). The FHA domain is believed to be a phospho-specific protein–protein interaction motif that recognizes the phosphorylated state of the target protein (Durocher et al. 2000). It was shown that the FHA/BRCT domain directly binds to γ-H2AX, the form of H2AX phosphorylated by ATM or ATR in the presence of DNA DSBs (double-strand breaks; Kobayashi et al. 2002), and recruits the R/M/N complex to the vicinity of sites containing DSBs. The FHA domain is also essential for the direct association of the R/M/N complex with chromatin (Zhao et al. 2002). Therefore, it seems reasonable to expect that the N-terminal region of NBS1 might have a role in determining the localization of the complex.

The C-terminal region of NBS1 binds to the MRE11/RAD50 complex. The MRE11-binding region at the NBS1 C-terminus, which consists of only ten amino acids, is well conserved from yeast to human (Tauchi et al. 2001). Figure 1 shows a comparison of the structures of NBS1 homologs reported from a wide variety of organisms. The NBS1 protein is widely found in eukaryotes such as fungi, insects, and vertebrates, whereas homologs in plants are unknown (Daoudal-Cotterell et al. 2002). A homolog in prokaryotes for the R/M/N complex is also unknown, although certain archaebacteria (e.g., *Pyrococcus furiosus*) do have homologs of MRE11 and RAD50 (Hopfner et al. 2000). This difficulty in finding potential homologs of NBS1 suggests that there might exist a wide diversity of amino acid sequences for this gene because diversity of the NBS1 amino acid sequence can be seen even in several higher vertebrates. For example, the entire amino acid sequence of chicken NBS1 shows only 48% identity to human NBS1, although the N-terminal 360 amino acids and C-terminal 124 amino acids are conserved (with 62% homology between chicken and human) (Tauchi et al. 2001). This is in sharp contrast to the other conserved members of the R/M/N complex, in which MRE11 shows a 78% identity between chicken and human.

Several SQ (or TQ) motifs, which are consensus sequences for phosphorylation by ATM or ATR kinases, are found in the central region of NBS1. In particular, the serine residues at 278 and 343 are phosphorylated by the ATM kinase in response to ionizing radiation both in vitro and in vivo, and such phosphorylation is responsible for

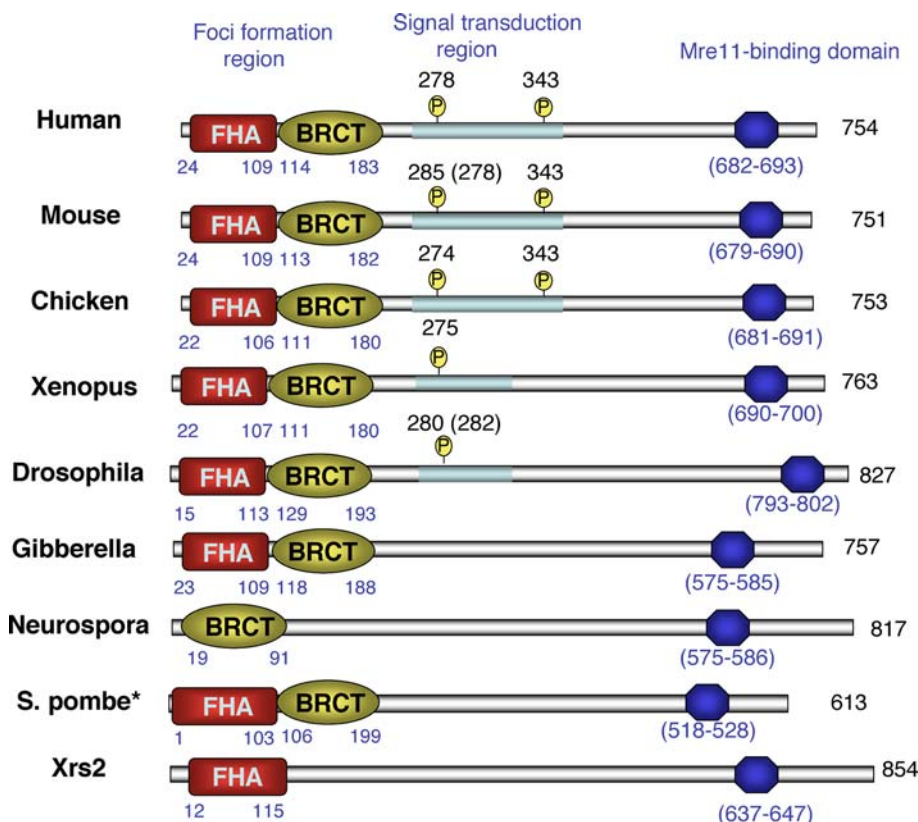

Fig. 1 Structure of NBS1 proteins derived from animals and fungus. All the NBS1 homologs have two conserved domains at their N-terminus (*FHA* or *BRCT*) and C-terminus (*Mre11 binding*). Animal NBS1 has a signal transduction region with SQ (or TQ) motifs, which can be phosphorylated by ATM or ATR kinases. The positions of the SQ motifs in chicken, *Xenopus* and *Drosophila* are predicted from their amino acid sequences. Several homologs of NBS1 from other mammals such as rat or bovine have also been sequenced and are similar to those from human or mouse. The figure shows NBS1 proteins for which the complete amino acid sequence has been reported, and only fragments of the proteins for fish NBS1 (*Fugu* and *Zebradanio*), which have also been reported (Ueno et al. 2003)

intra-S-phase checkpoint control (Lim et al. 2000; Zhao et al. 2000; Wu et al. 2000a). These serine residues are well conserved in vertebrates (Tauchi et al. 2001), although only the serine 278 consensus motif is conserved in animals or insects such as *Xenopus* and *Drosophila*. No phosphorylation motifs have been identified yet in yeast or fungus (Fig. 1). These evolutionary aspects suggest that phosphorylation of NBS1 by ATM/ATR kineses might have been acquired after the appearance of multicellular animals, possibly in order to adapt the complex to a specific regulatory role or function.

Function of NBS1 complexes in DNA damage checkpoints

NBS1 is a regulatory subunit of the R/M/N complex and functions in the localization of the complex (Dong et al. 1999), in signal transduction and in catalytic activation (Mirzoeva and Petrini 2001; Desai-Mehta et al. 2001). NBS cells exhibit defects in cell-cycle checkpoints after exposure to ionizing radiation or treatment with radiomimetic drugs. One of the well-characterized checkpoint defects is RDS (radiation-resistant DNA synthesis) in which NBS cells continue DNA replication in the presence of radiation-induced DNA damage (Painter and Young 1980). Radiation-resistant DNA synthesis is due to the failure of the intra-S-phase checkpoint to inhibit or reduce ongoing DNA synthesis. The extent of RDS in NBS cells is milder than that in cells deficient in ATM, suggesting that the intra-S-phase checkpoint is regulated by two parallel branches that are triggered by ATM (Falck et al. 2002). One branch involves the Chk2–Cdc25A pathway (Falck et al. 2001) and the other requires NBS1. The S-phase checkpoints that require NBS1 involve a key protein, SMC1. The SMC1 is phosphorylated in response to agents causing DNA damage, such as ionizing radiation, hydroxyurea and UV (Yazdi et al. 2002; Kim et al. 2002). However, this phosphorylation does not occur in either NBS cells or AT cells after exposure to radiation, since serines 957 and 966 of SMC1 are substrates for the ATM kinase, and this phosphorylation requires the phosphorylation of NBS1 on both serines 278 and 343. These observations clearly indicate that both ATM and NBS1 are involved in intra-S-phase checkpoint controls through the phosphorylation of SMC1. Phosphorylation of SMC1 may interfere with the function of cohesin between the template and the sister chromatid undergoing replication. ATLD cells, with mutations in the MRE11 gene, also show RDS (Stewart et al. 1999) and hence the R/M/N complex is involved in intra-S-phase checkpoint control.

NBS1 could also be involved in G1 checkpoint function (Jongmans et al. 1997; Matsuura et al. 1998; Yamazaki et al. 1998; Antoccia et al. 1999) and G2 checkpoint regulation (Buscemi et al. 2001; Williams et al. 2002) after exposure to ionizing radiation. The impairment of the G2 checkpoint is due to a delay in Chk2 phosphorylation, and the introduction of full-length NBS1 into NBS cells restores this delay to the same interval seen in wild-type cells (Buscemi et al. 2001). Girard et al. (2002) demonstrated a dose dependence for these checkpoint controls. Defects in the G1 checkpoint and also in the induction of p53/p21 are observed in NBS cells exposed to low doses, but this is not apparent in cells exposed to high doses of ionizing radiation. ATM, a kinase responsible for the phosphorylation of Chk2 and p53 after irradiation, is activated through autophosphorylation at serine 1981 and subsequent dimer dissociation, which is induced by changes in the structure of chromatin caused by DSBs (Bakkenist and Kastan 2003). The activation of ATM after treatment with radiomimetic agents or low-dose irradiation is delayed in NBS cells (Carson et al. 2003; Horejsi et al. 2004). In addition, phosphorylation levels of several ATM substrates, such as p53 and Chk2, are decreased and delayed in NBS cells (Matsuura et al. 1998; Buscemi et al. 2001). These observations suggest that ATM may require the R/M/N complex not only for its efficient activation but also for efficient phosphorylation of substrates after DNA damage induction. In fact, recent in vitro assays by Lee and Paull (2004) indicated that the ATM kinase is directly activated by the R/M/N complex, and when it binds to the R/M/N complex, ATM then seems to bind to substrates with a high affinity (Lee and Paull 2004). These recent reports suggest that NBS1 is not only a substrate of ATM but also an upstream regulator of ATM activity. This provides us with an explanation for why AT cells, NBS cells and ATLD cells share common cellular features. Recently, it has been found that NBS1 is involved in an apoptosis-inducing pathway that is independent of p53 phosphorylation (Tauchi et al., unpublished observation), suggesting the possible existence of another NBS1-regulated damage responding pathway, which is independent of ATM. This could be a reason why malignancy in NBS patients is more pronounced than in AT patients (Shiloh 1997) since this pathway could prevent tumorigenesis through the exclusion of abnormal cells by apoptosis.

Role in DNA damage repair

Double-strand DNA breaks, often induced by ionizing radiation, represent the most serious type of damage that can occur among the variety of possible DNA-damaging events or products. There are at least two pathways to rejoin DSBs: non-homologous end joining (NHEJ) and homologous recombination (HR) repair. Proteins for NHEJ (such as Ku70/80, DNA-PKcs, XRCC4, and DNA ligase IV) and proteins for HR (such as RAD51, RAD52, RAD54) are highly specific for each DSB-rejoining pathway, although the yeast Xrs2 complex (R/M/X complex) is involved in both pathways (Haber 1998). The yeast *xrs2* mutant displays hypersensitivity to ionizing radiation, due to insufficient NHEJ and HR activity, as well as a deficiency in meiotic recombination (Ohta et al. 1998). In higher vertebrate cells, it has been shown that NBS1 functions in HR repair rather than NHEJ. We demonstrated that NBS1-deficient chicken DT40 cells are

viable, whereas NBS1-null mouse cells are not. The chicken NBS1-disrupted cells show a dramatic reduction in mitomycin C-induced sister chromatid exchange. This is quite similar to the phenotypes of other mutants deficient in HR proteins, such as RAD54 and RAD51 paralogs (Sonoda et al. 1999; Takata et al. 2000). It has also been observed that HR-mediated target integrations at specific loci are dramatically reduced in NBS1-deficient chicken cells, and HR events quantified using the SCneo reporter gene assay are reduced approximately 250-fold (Tauchi et al. 2002). This is consistent with the reported observation that H2AX-knockout mice, which are defective in localizing NBS1 to the sites of DSBs, are also defective in meiotic recombination and HR repair (Celeste et al. 2002; Bassing et al. 2002).

On the other hand, NHEJ events in NBS1-disrupted chicken cells are indistinguishable from those in wild-type DT40 cells, indicating that NBS1 is not essential for conventional NHEJ (Tauchi et al. 2002). This is supported by evidence that V(D)J recombination, which is known to be mediated by NHEJ, is quite normal in NBS patients (Harfst et al. 2000). The R/M/N complex is required for Ig class switch recombination (van Engelen et al. 2001; Petersen et al. 2001) and this might explain why NBS patients are immunodeficient. The class switch pathway is mediated by NHEJ proteins (Manis et al. 1998) and also mismatch-repair proteins such as MLH1, MSH2 and PMS2 (Ehrenstein et al. 2001). Therefore, it is possible that impairment of class switch recombination in NBS patients could be associated with mismatch-repair deficiency, since mismatch repair-deficient cancer shows reduced expression of the R/M/N complex, and loss of foci formation (Giannini et al. 2002).

Although the R/M/N complex is not essential for NHEJ, data obtained with *NBS1*-mutant mice suggest an indirect involvement of NBS1 in V(D)J recombination. When V(D)J recombination occurs at TCR-β and/or TCR-γ loci, inter-chromosomal V(D)J recombination is increased in *NBS1*-mutant mice when compared with normal mice, whereas intra-chromosomal recombination is not significantly affected (Kang et al. 2002; Williams et al. 2002). This is consistent with the frequent chromosomal translocations observed at TCR loci in NBS patients. These findings suggest that NBS1 plays a role in recognizing the proper elements of V(D)J recombination by suppressing inter-chromosomal recombination. This suppressive role of NBS1 in inter-chromosomal recombination is also observed in HR events. Significant inter-chromosomal recombination has been observed in NBS1-deficient cells when HR repair activity was examined using the SCneo reporter gene assay (Tauchi et al. 2002). In yeast, *xrs2* mutants also showed an approximately 100-fold higher chromosomal rearrangement frequency when compared with that seen in yeast *rad52* or *ku70* mutants. Formation of these rearrangements may involve the initiation of break-induced replication using another chromosome as template (Chen and Kolodner 1999). It is known that SMC-family proteins are involved in the bridging of distal sites on DNA, and in events such as forming cohesion between chromatids or in condensation of DNA. Therefore, RAD50, an SMC-like protein, which binds to DNA-break ends could help tether linear DNA at sites of DSBs (de Jager et al. 2001; Hopfner et al. 2002). Thus, NBS1 seems to be essential for suppression of inter-chromosomal NHEJ and HR through R/M/N complex formation, and abrogation or reduction of complex formation or function could cause genomic instability, such as translocations, as observed in NBS cells.

During the proper processing of DSBs, the R/M/N complex associates with many DNA repair-related proteins such as ATM, MDC1, SMC1, BRCA1, p53BP1, FANCD2, and WRN. In these interactions, NBS1 plays a key role, i.e., all the associations are mediated by the ability of NBS1 to interact with the target proteins. For example, NBS1 mediates association between the R/M/N complex and WRN, the RecQ helicase that is responsible for Werner syndrome, through direct protein–protein interaction (Cheng et al. 2004). This association is enhanced by DNA damage, and the co-localization of R/M/N with WRN foci can be seen 4–8 h after radiation exposure. This association promotes in vitro helicase activity but not the exonuclease activity of WRN (Cheng et al. 2004). Because the association between WRN and R/M/N also occurs in response to stalled replication forks (Pichierri and Franchitto 2004), R/M/N-WRN could function not only for the proper processing of recombination, but also to prevent any DNA replication forks from collapsing or failing, which could lead to genome instability.

Relocation of NBS1 complexes responding to DNA damage

Many proteins that are involved in response to DNA damage form nuclear foci when cells are exposed to DNA-damaging agents such as ionizing radiation. The R/M/N protein complex is one of these, and forms nuclear foci at DSB sites. In live human cells with DSBs restricted to small sub-nuclear areas, NBS1 is rapidly recruited to the damaged regions and undergoes a dynamic exchange in the close vicinity of the DSB sites (Lukas et al. 2003). Rogaku et al. (1998) reported that the serine residue of the SQ motif in the C-terminus of H2AX becomes phosphorylated and that phosphorylated H2AX (γ-H2AX) foci are formed in response to radiation- and chemically induced DSBs. H2AX phosphorylation and foci formation are apparent within a few seconds after irradiation and reach a maximum 10–30 min after irradiation, followed by degradation, with a half-life of about 2 h (Kobayashi et al. 2002). ATM has been suggested to be an early kinase for γ-H2AX responding to irradiation, although other PI-3 kinases, such as ATR, might act as a kinase for this phosphorylation, especially in S-phase (Burma et al. 2001; Ward and Chen 2001). MDC1, a mediator of a DNA-damage checkpoint and a substrate of ATM, might be responsible for the promotion of γ-H2AX foci formation because siRNA depletion of MDC1 significantly affects

the phosphorylation and foci formation ability of γ-H2AX (Goldberg et al. 2003; Stewart et al. 2003). Possibly the mode of action of γ-H2AX is to modify chromatin to allow access by repair factors for the rejoining of DSBs because yeast mutant H2A containing the mutation 129Ser/Glu, for a constitutively phosphorylated form of the mammalian H2AX homolog, exhibits decondensation of chromatin (Down et al. 2000). The FHA domain of NBS1 functions in direct protein–protein interaction with γ-H2AX to form the R/M/N foci (Kobayashi et al. 2002). This is consistent with the fact that NBS1 foci formation after DNA damage is impaired in H2AX-disrupted cells (Celeste et al. 2002). H2AX-disrupted cells are hypersensitive to ionizing radiation and display reduced homologous recombination (Celeste et al. 2002; Bassing et al. 2002). Interestingly, the R/M/N complex could localize itself to a damage site in the absence of H2AX when a clustered damage region was introduced using a laser microbeam on the cell nucleus (Celeste et al. 2003). Even in this case, however, the localized R/M/N complex could not form "normal foci" at the damage sites. These observations suggest that the interaction between NBS1 and γ-H2AX is required for nuclear foci formation, which then activate suitable DNA damage responding pathways. The R/M/N complex also co-localizes with foci of BRCA1, 53BP1, and MDC1 at least part of the time (Goldberg et al. 2003; Stewart et al. 2003), but not with RAD51, which plays a central role in HR and foci formation (Nelms et al. 1998). Therefore, it is still unclear how the R/M/N foci are directed to the proper locations by the HR complex components involved in DNA strand invasion since this includes RAD51. Surprisingly, the FHA domain of NBS1 is not essential for ATM activation whereas the MRE11-binding domain of NBS1 is critical (Horejsi et al. 2004). Because the FHA/BRCT domain of NBS1 is required for complementation of radiation sensitivity, normal S-phase checkpoint function, and direct association with chromatin in NBS cells (Zhao et al. 2002), those domains might act in the steps that follow nuclear foci formation.

Nuclear body and NBS1 complex formation

NBS1 could have a role in telomere maintenance because primary cells from NBS patients display accelerated telomere shortening during replication (Ranganathan et al. 2001). In fact, NBS1 physically interacts with the telomere-binding proteins, TRF1 and TRF2, at mammalian telomeres (Zhu et al. 2000; Wu et al. 2000b). The reduced telomere lengths in NBS cells are restored to normal levels only after the co-expression of NBS1 and hTERT (Ranganathan et al. 2001). This evidence implies that the role of the R/M/N complex in telomere maintenance is to generate G-strands (telomeric 3′-overhangs), and hTERT can then replicate the telomeres by using this G-strand as a primer. On the other hand, it has been reported that telomere length in telomerase-negative cancer cells is maintained by an alternative mechanism: alternative lengthening of telomeres or ALT. The telomere-associated nuclear body, which is dependent on the promyelocytic leukemia protein (PML), is often found in the nucleus of ALT cells. These PML nuclear bodies probably function in the process of telomere lengthening

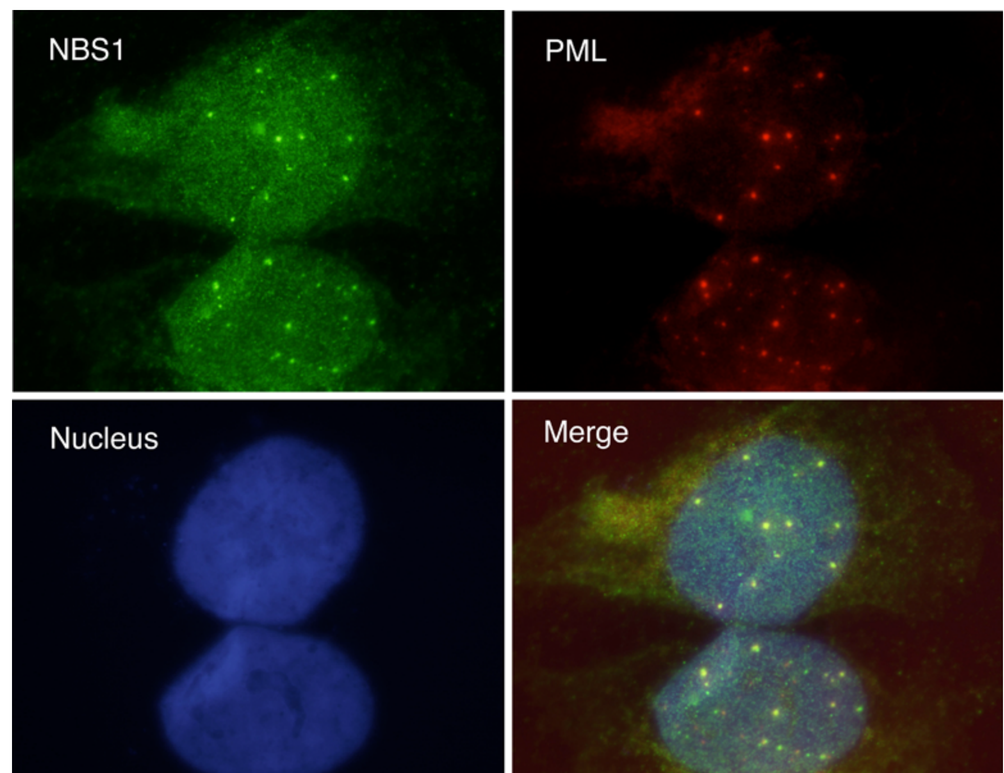

Fig. 2 Colocalization of NBS1 in telomere-associated promyelocytic leukemia protein (*PML*) body in ALT (alternative lengthening of telomeres) cells. KMST-6 human cells were fixed with methanol, and PML and NBS1 were detected using anti-PML monoclonal antibody and anti-NBS1 polyclonal antibody, respectively

through recombination, because they appear at exactly the same time as the activation of the ALT mechanism during cell immortalization, and because these nuclear bodies include RPA, RAD51, and RAD52 (Yeager et al. 1999). NBS1 is also included in the ALT-associated PML nuclear body (APB) (Wu et al. 2000b). In ALT cells, clear NBS1 foci can be observed in the nucleus from G1- to G2-phase, and they colocalize with the PML body (Fig. 2). However, the PML bodies co-localize with telomeres only during G2-phase, suggesting that the APBs might be formed only when they act during telomere maintenance. SP100, which is a nuclear dot-associated PML-binding protein, interacts with the BRCT domain of NBS1 and recruits NBS1 into the APBs (Naka et al. 2002). The NBS1 protein then recruits other recombination factors such as RAD50/MRE11 and BRCA1, but not RAD51 or TRF1 although these two proteins are also involved in APBs (Wu et al. 2003). This observation suggests that APBs could have a role in telomere elongation via a process similar to that of the HR repair of DSBs, and that NBS1 plays a key role in the assembly of the APB-related factors in ALT cells. MRE11–NBS1–PML co-localization is also found at meiotic telomeres (Lombard and Guarente 2000), suggesting a specific function of the NBS1 complex in elongating the telomeric repeats, not only in ALT cells but also in normal germ cells.

NBS1 and MRE11 are concentrated in the PML nuclear body in the absence of DNA damage in normal primary cells. The short-term dissociation of R/M/N from the PML nuclear body occurs after irradiation (Lombard and Guarente 2000). This suggests that the role of the PML nuclear body is to serve as a reservoir for proteins that can translocate to the damaged site to repair damage or to activate checkpoints. Subsequently, significant association between the PML nuclear body and the R/M/N complex can be found at ionizing radiation-induced foci around 8–12 h after irradiation. These PML-including R/M/N foci then associate with p53 from 12 h to 24 h after irradiation (Carnone et al. 2002), suggesting that PML-including foci could be related to the late stage of a DNA-damage response.

Conclusions

Double-strand ends of DNA must be properly processed to maintain genomic stability. NBS1 is a member of the R/M/N complex, and this complex has multiple functions in the processing of two types of DNA double-strand ends, i.e., DSBs and telomeres. The R/M/N complex activates ATM kinase in response to DSB induction by ionizing radiation. Subsequently, together with ATM, it regulates cell-cycle checkpoints, DNA repair via homologous recombination, and apoptosis induction, by activating numerous signal transducers and mediators (Fig. 3). NBS1 is required not only for the promotion of enzymatic activity of the R/M complex, but also for the relocation of the complex. NBS1 regulates the association between the R/M/N complex and other suitable partner proteins to allow them to function properly. For DSB processing, NBS1 interacts with γ-H2AX to form nuclear foci, which may be essential for a normal DNA damage response. NBS1 also interacts with TRF2 to process the telomere T-loop, or with PML-SP100 in order to elongate telomeric repeats via the ALT mechanism. In addition, the NBS1 complex localizes at ALT-independent PML nuclear bodies, which could be a reservoir of repair-related proteins, which then translocate to a damaged DNA site in response to DSBs. The reported functions of NBS1 described here indicate that NBS1 is a key regulator of sensor-mediator proteins that respond to DNA double-strand ends, both those induced by damaging agents such as ionizing radiation, or present as a part of normal physiological processes. These NBS1 functions then

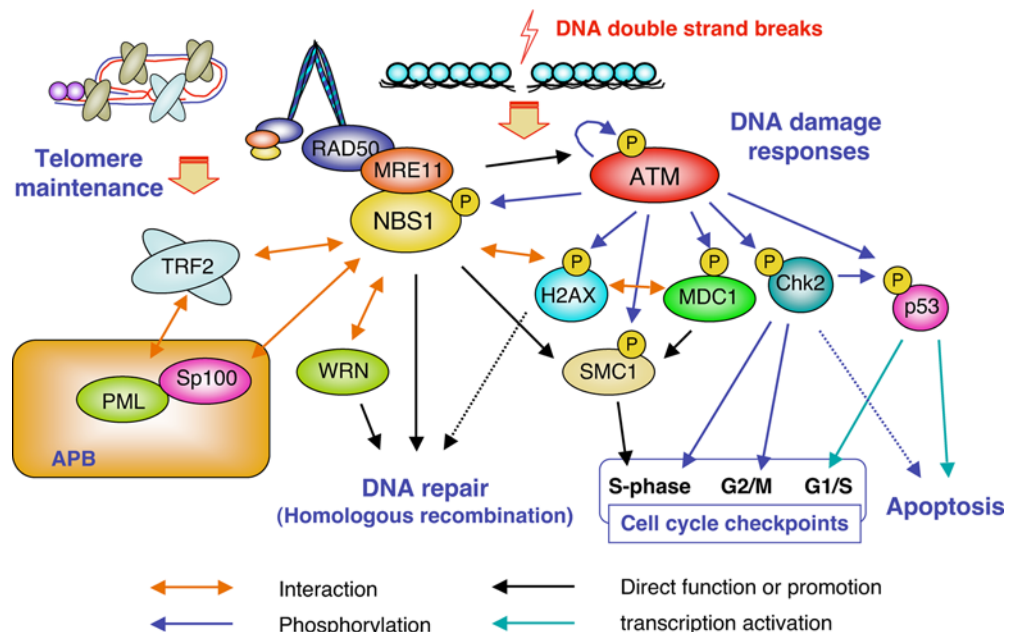

Fig. 3 NBS1-regulated pathways for the maintenance of genomic stability. NBS1 from the R/M/N complex regulates processing pathway functions in two types of DNA double-strand ends, i.e., in DNA breaks and in telomeres. *Arrows* indicate activity of the proteins in signal transduction that responds to the presence of DNA double-strand ends. Note that the R/M/N complex could consist of four molecules each of RAD50, MRE11, and NBS1 (Lee et al. 2003), although only two molecules of each are shown

enable the cell to maintain its DNA integrity and genomic stability.

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