available at www.sciencedirect.com

SCIENCE DIRECT®

journal homepage: www.elsevier.com/locate/dnarepair

# A novel function of DNA repair molecule Nbs1 in terminal differentiation of the lens fibre cells and cataractogenesis☆

*Yun-Gui Yang*[a,1], *Pierre-Olivier Frappart*[a,2], *Lucien Frappart*[b], *Zhao-Qi Wang*[a,3], *Wei-Min Tong*[a,*]

[a] *International Agency for Research on Cancer (IARC), 150 cours Albert-Thomas, 69008 Lyon, France*
[b] *Department of Pathology, Hospital Edouard Herriot, 69003 Lyon, France*

ARTICLE INFO

*Article history:*
Received 9 February 2006
Received in revised form 2 May 2006
Accepted 2 May 2006
Published on line 21 June 2006

*Keywords:*
Nbs1
DNA repair
Terminal differentiation
Cataract

ABSTRACT

The Nbs1 protein, hypomorphic mutant in Nijmegen breakage syndrome (NBS), is a component of the Mre11/Rad50/Nbs1 (M/R/N) complex that acts as a DNA double-strand break sensor and functions in cell cycle checkpoint in response to DNA damage and DNA repair. Here we report that targeted disruption of murine *NBS1* gene (*Nbn*) in the lens alters the M/R/N complex nuclear localization and results in microphthalmia in mice due to reduced proliferation of the lens epithelial cells. Unexpectedly, all *Nbn*-deficient lenses develop cataracts at an early age due to altered lens fibre cell differentiation, including disruption of normal lens epithelial and fibre cell architecture and incomplete denucleation of fibre cells, and these changes are independent of the p53 pathway. In addition, *Nbn*-deficient lenses show dysregulated transcription of various crystallins. Thus, this study implicates a novel function of Nbs1 in terminal differentiation of the lens fibre cells and in cataractogenesis.


## 1. Introduction

Nijmegen breakage syndrome (NBS), caused by mutations in the *NBS1* gene, is an autosomal recessive chromosomal instability syndrome characterized by cancer predisposition. Cells isolated from NBS patients display increased levels of spontaneous chromosome aberrations and sensitivity to ionising radiation [1]. The Nbs1 protein, hypomorphic mutant in NBS, is a component of the Mre11/Rad50/Nbs1 (M/R/N) complex that harbors nuclease, DNA binding and DNA end recognition activities [2,3]. The M/R/N complex acts as a DNA double-strand break (DSB) sensor and functions in cell cycle checkpoint in response to DNA damage and DNA repair in concert with Ataxia telangiectasia mutated (ATM), Ataxia telangiectasia and Rad3-related (ATR), MDC1 and H2AX [4]. Nbs1 has been shown to directly regulate ATM and ATR activation in response to DNA strand-breaks to induce cell cycle arrest and facilitate DNA repair [5–7].

Repair of damaged DNA is essential in the maintenance of genomic integrity and development, and defects in DNA repair may lead to fatal disease such as developmental abnormalities, chromosomal instability syndromes or cancer [2,3]. Chromosomal instability syndromes such as Ataxia telangiectasia (AT) [8,9], Fanconi anemia [10] and Cockayne syndrome [11] are predisposed to cataracts. In addition, individuals with premature aging syndromes associated with

☆ In the mouse, the recognized symbol for the gene encoding Nbs1/nibrin is *Nbn*.

* *Corresponding author*. Tel.: +33 4 7273 8436; fax: +33 4 7273 8698.
E-mail address: tong@iarc.fr (W.-M. Tong).
[1] Present address: Clare Hall Laboratories, Cancer Research UK, South Mimms, Hertfordshire EN6 3LD, UK.
[2] Present address: Department of Genetics and Tumor Cell Biology, St Jude Children's Research Hospital, Memphis, TN 38105-2794, United States.
[3] Present address: Lebniz Institute for Age Research-Fritz Lipann Institute e.V., 07745 Jena, Germany.
1568-7864/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.dnarep.2006.05.004

RecQ helicase deficiency, such as Werner's syndrome and Rothmund–Thomson syndrome, have also been associated with cataracts [12]. These observations suggest that defects in DNA damage response (DDR) and repair pathways may be a risk factor for cataractogenesis.

Cataract is one of the commonest causes of loss of vision worldwide. Risk factors include increasing age, smoking, diabetes, and oxidative damage, as well as hereditary mutations in crystallin and connexin genes affecting lenses function [13–15]. The eye lens is composed of a single layer of epithelial cells on the anterior surface of the lens that differentiates into quiescent, and structurally highly differentiated fibre cells that are characterized by changes in cell shape, expression of crystallins and the degradation of cellular organelles, hence ensuring the lens transparency [16]. Disorganization of the cells and alterations in the cytoplasm within the epithelium and fibre, and defects in terminal differentiation of the lens fibres all lead to the development of cataract in mammals [14,17–19].

To investigate the possible role of DDR in cataractogenesis, we used mice carrying conditional neural-specific deletion of the *Nbn* allele modified by *nestin-Cre*-mediated excision of exon 6 flanked by two *lox*P sites ($Nbn^{F6/F6}$*nestin-Cre+*, designated $Nbn^{CNS\text{-}del}$) [20], because the *nestin* gene is expressed in mouse lens epithelium, fibre cells and neuroretina during lens development [21]. We have shown previously that neuronal-specific deletion of *Nbn* in mice resulted in severe brain developmental defects, growth retardation and ataxia [20]. Using this in vivo model, we report here that deletion of *Nbn* in mice causes microphthalmia and cataract due to reduced proliferation of the lens epithelial cells and altered lens fibre cell differentiation. This result implies a novel function of Nbs1 in lens development. This finding may have significant implications for defects in DNA repair molecules as risk factors for human cataractogenesis.

## 2. Materials and methods

### 2.1. *Nbs1 mutant mice and genotyping*

The generation and genotyping of $Nbn^{F6/F6}$*;nestin-Cre+* (designated $Nbn^{CNS\text{-}del}$) mice has been described previously [20]. All *nestin-Cre* positive mice in $Nbn^{F6/+}$ and *Nbn+/+* background, and *nestin-Cre* negative mice in $Nbn^{F6/F6}$, $Nbn^{F6/+}$, and *Nbn+/+* background were grouped as controls. The *Trp53−/−* [22] and $Nbn^{F6/+}$*nestin-Cre+* mice were interbred to generate $Nbn^{F6/+}$*nestin-Cre+Trp53+/−* mice, which were intrcrossed to generate $Nbn^{F6/F6}$*nestin-Cre+Trp53−/−* ($Nbn^{CNS\text{-}del}$*Trp53−/−*) mice. Genotyping for *Trp53* loci was performed as described [23]. All animal experiments were approved by and performed in accordance with the guidelines of the International Agency for Research on Cancer's Animal Care and Use Committee.

### 2.2. *Histological and electron microscopic analyses of eye lenses*

For histological analysis, mouse lenses were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2), and embedded in paraffin. The sections (3 μm) were subjected to hematoxylin/eosin (H&E) staining. To visualize the fibre nuclear alterations, sections were stained with 4,6-diamidino-2-phenylindole (DAPI, Vector Laboratories Inc., Burlingame, CA). For electron microscopy, the lenses from three-week-old mice were fixed with 4% glutaraldehyde in 0.2 M phosphate buffer (pH 7.4), post-fixed with 2% OsO4 at 4 °C for 1 h, and embedded in Epon. Ultra-thin sections (80 nm) were stained with lead citrate and uranyl acetate, and observed under a JEOL 100 CX electron microscope (JEOL SA, Reuil Malmaison, France). For embryonic lens proliferation analysis, pregnant females were injected intraperitonally with 5-bromo-2′-deoxyuridine (BrdU, 50 μg/g of body weight, Sigma, St Louis, MO) at E13.5 to E18.5, and embryos were collected 6 h after the injection and were fixed with 4% paraformaldehyde. Antibodies against nestin (Chemicon International), Mre11 (Novus Biologicals), and BrdU (Sigma) were used for immunostaining as described previously [20]. Quantification was performed by scoring the percentage of BrdU-positive lens epithelial cells from four serial sections of each mouse and a total of four control and Nbs1-deficient mice, respectively, were examined.

### 2.3. *RT-PCR analysis*

Lenses from 8 to 10 $Nbn^{CNS\text{-}del}$ or control mice at 2–3 weeks old were dissected, pooled for total RNA extraction using Tri-Reagent (Sigma) and 1 μg total RNA was used for cDNA synthesis using Invitrogen RT-PCR kit. Semiquantitative PCR for *Nbn, Mre11 and Rad50* transcripts using 1 and 1/10 cDNA template, for α-, β- and γ-crystallin mRNA using 1, 1/2, 1/5 cDNA template were analysed as described previously [24,25]. *hprt* was used as an internal control. Primer sequences are summarized in Supplementary Table 1.

Serial dilutions of cDNA templates from Nbs1-deficient and control lenses were used to confirm that PCR was performed within a linear range. Relative expression of *Nbn* and crystallin mRNA in Nbs1-deficient lenses as compared with Nbs1-proficient lenses was determined by normalizing with the *hprt* internal control at each gradient to obtain the means and standard deviations.

## 3. Results

### 3.1. *Expression of Nbs1/Mre11/Rad50 in the lens*

We first examined the efficiency of the *Nbn* expression and deletion in $Nbn^{CNS\text{-}del}$ lenses by PCR with reverse transcription (RT-PCR). As lenses undergo denucleation during terminal differentiation [16], only a small portion of cells, including the epithelial cells and subcapsular nucleated fibre cells, have nuclei. Therefore, in order to obtain sufficient RNA, we pooled lenses from 8 to 10 $Nbn^{CNS\text{-}del}$ or control mice at 2–3 weeks of age. Using a pair of primers between exons 5 and 7 of *Nbn*, we found *Nbn* transcription in wild type lenses (Fig. 1A). In addition, *Mre11* and *Rad50* are also transcribed in wild type lenses (Fig. 1A), indicating a putative function of the M/R/N complex in the lens. In the $Nbn^{CNS\text{-}del}$ lenses, a significant reduction (95% knock-down) of *Nbn* mRNA was observed (Fig. 1A), implying that the Cre recombinase results in effective down-regulation of the *Nbn* gene in the lens. Interestingly, levels

of *Mre11* and *Rad50* mRNA expression were similar between control and *Nbn*-deficient lenses (Fig. 1A), suggesting that loss/decrease of one component of the M/R/N complex does not affect expression of others. We then confirmed expression of nestin that mediates Cre recombinase in the lens epithelial and fibre cells (Fig. 1B). Although we failed to obtain specific Nbs1 staining using several antibodies, nuclear localization of the Nbs1 partner Mre11 protein was evidenced in the lens

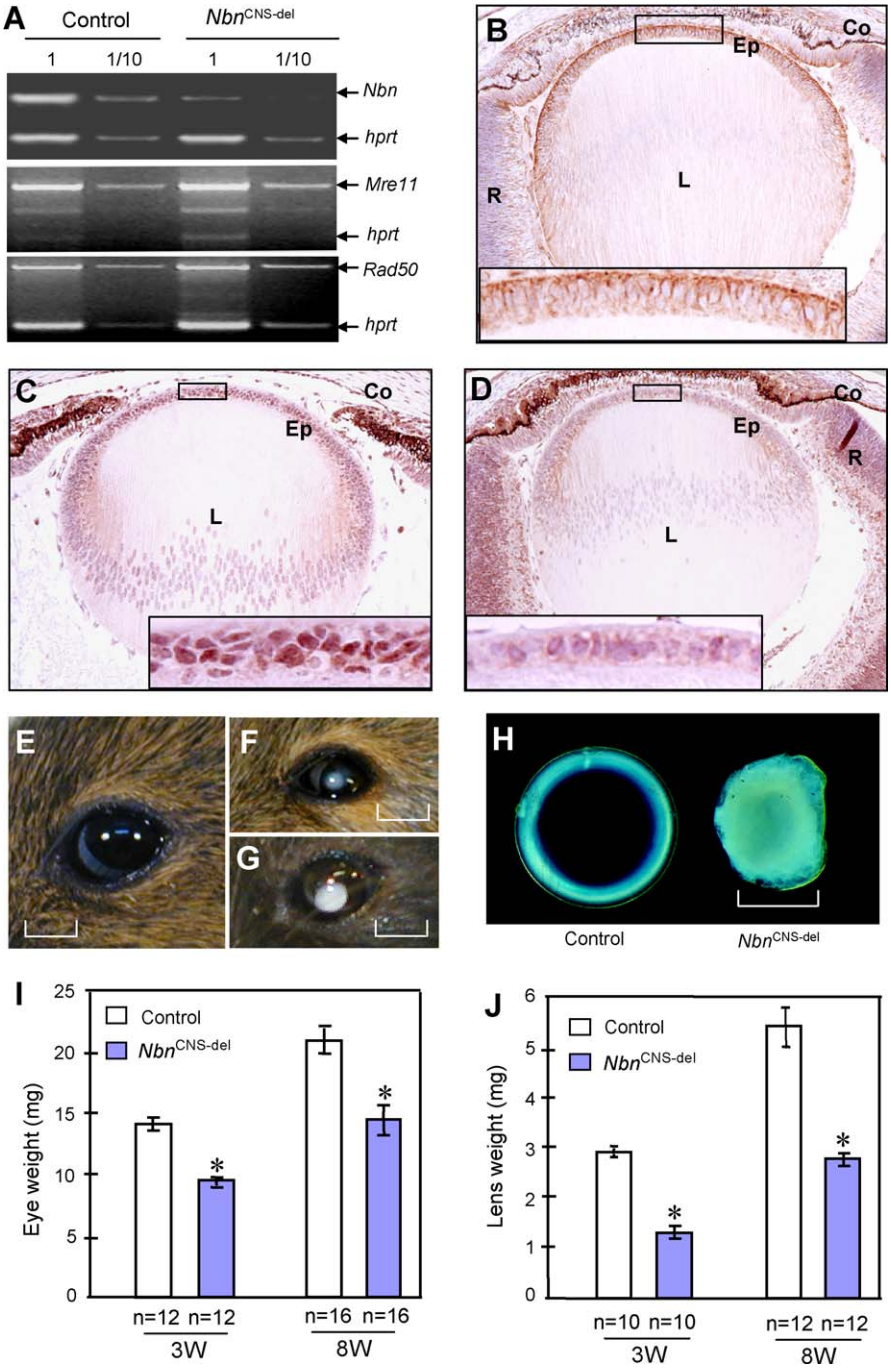



**Fig. 1 – Microphthalmia and cataracts in *Nbn*$^{CNS\text{-}del}$ mice. (A) RT-PCR analysis of *Nbn*, *Mre11* and *Rad50* mRNA in lenses of *Nbn*$^{CNS\text{-}del}$ mice and controls. The genotypes and cDNA template dilutions are indicated at the top of lanes. *Hprt* was used as an internal control. (B) Representative nestin expression in the embryonic day 18.5 (E18.5) lens. Immunostaining of Mre11 in controls (C) and in *Nbn*$^{CNS\text{-}del}$ lenses (D). High magnification of the rectangular regions in B–D is shown in inserts. *Note*: Ep, lens epithelium; R, retina; L, lens; Co, cornea. Original magnification (B–D): × 20. (E) A normal eye from a 4-week-old control mouse. Representative microphthalmia and opacity observed in a 4-week-old (F) and a 12-week-old (G) *Nbn*-deficient eye. (H and I) *Nbn*$^{CNS\text{-}del}$ mice show microphthalmia at 3 and 8 weeks (W) of age. (F–H and J) *Nbn*$^{CNS\text{-}del}$ lenses show opacity and are smaller in weight compared to the controls. Bars in E-G are 2 mm, in H is 1 mm. $^{*}p < 0.001$, *t* test.**

epithelial cells (Fig. 1C), and cytoplasmic Mre11 was observed during denucleation of the lens fibre cells in wild-type mice (Fig. 1C). However, consistent with a previous report [26], loss of Nbs1 protein disrupted nuclear localization of Mre11 in the lens epithelial and fibre cells (Fig. 1D), suggesting an alteration of the M/R/N complex function in the lenses.

### 3.2. *Nbn-deficient mice develop microphthalmia and cataracts*

Beginning around 2 weeks of age, most of the *Nbn*$^{CNS\text{-}del}$ mice exhibited microcornea and microphthalmia (Fig. 1F–G) compared with controls (Fig. 1E). The eyeballs and lenses of

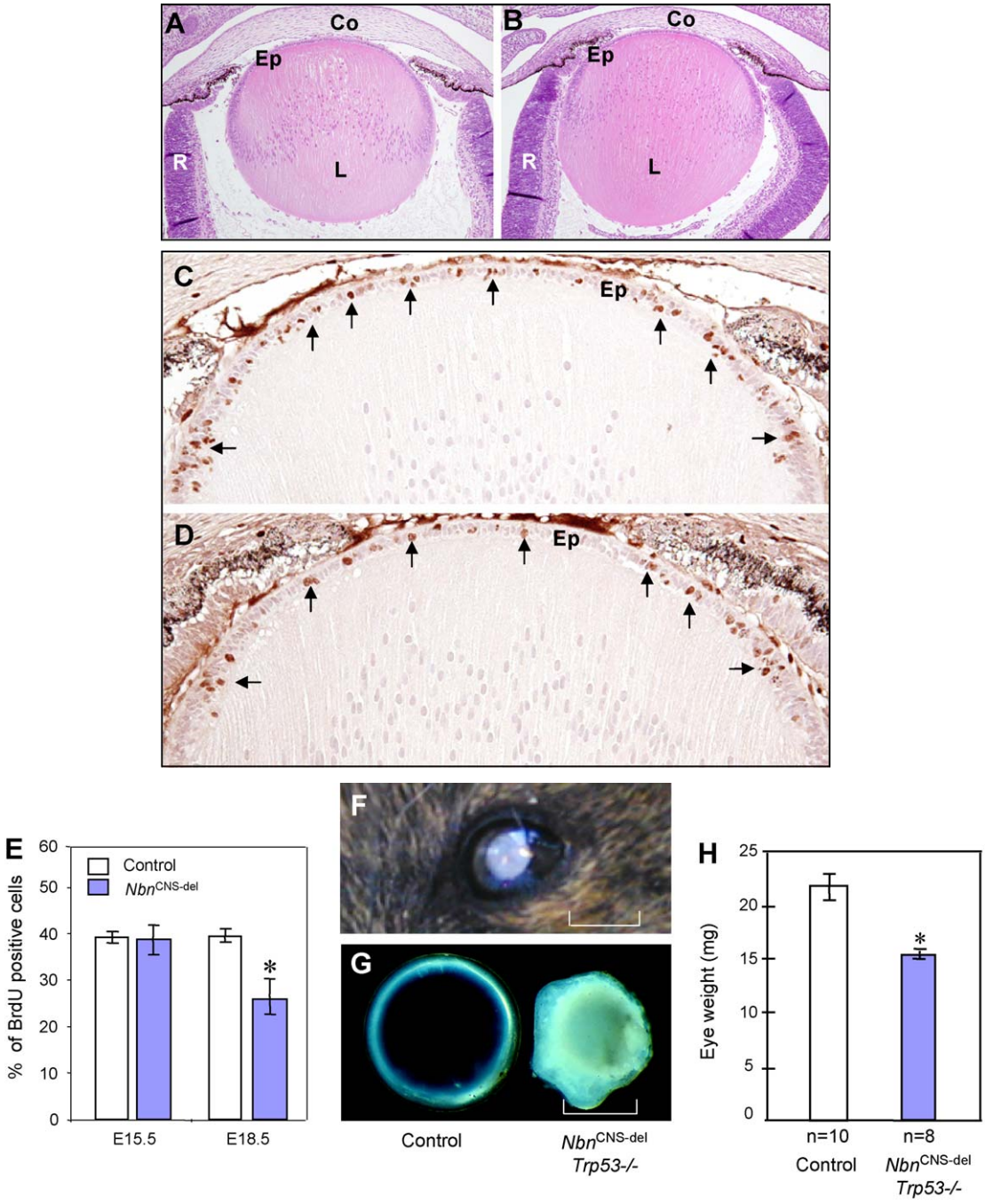



**Fig. 2 – Nbs1 deficiency results in proliferation defects of epithelial cells in developing lenses independent of p53. Hematoxylin and eosin (H&E) staining of a representative control (A) and *Nbn*$^{CNS\text{-}del}$ lens (B) at E18.5. *Note*: Co, cornea; Ep, epithelium; L, lens; R, retina. Original magnification: × 10. (C–D) Representative in vivo BrdU labelling shows a lower number of BrdU-positive cells (arrows) in the lens epithelium (Ep) of E18.5 *Nbn*$^{CNS\text{-}del}$ embryos (D) compared to the littermate control (C). (E) Quantification of BrdU-positive cells in the lens epithelium from four mice of each genotype ($^{*}p < 0.001$, *t* test). *Nbn*$^{CNS\text{-}del}$;*Trp53*−/− eyes show opacity (F–G) and are smaller in the lens weight compared to the controls in 12-week-old mice (H). Bars in F is 2 mm, in G is 1 mm. $^{*}p < 0.001$, *t* test.**

*Nbn*$^{CNS\text{-}del}$ mice were significantly smaller than those of control mice after 2 weeks of age (Fig. 1H–J). Strikingly, *Nbn*$^{CNS\text{-}del}$ mice exhibited abnormal light scattering of the lenses at about 3 weeks of age, and its severity increased with age (Fig. 1F–H). Bilateral opacity (cataract) was evident in all *Nbn*$^{CNS\text{-}del}$ lenses ($n = 156$) between 4 and 6 weeks of age in mice of 129/SV × C57/BL/6 background derived from two independent embryonic stem cell clones. Out of more than 900 controls, including all *nestin-Cre* positive mice in *Nbn*$^{F6/+}$ and *Nbn*+/+ background, and *nestin-Cre* negative mice in *Nbn*$^{F6/F6}$, *Nbn*$^{F6/+}$, and *Nbn*+/+ background, no microphthalmia and cataracts were observed during nine months of monitoring.

To define the onset and possible cause of microphthalmia, we performed histological analysis on the embryonic lenses at embryonic (E) days 13.5, 15.5 and 18.5, and found no apparent morphological difference in the lens and neuronal retina between the *Nbn*$^{CNS\text{-}del}$ and control mice at any of these stages (Fig. 2A–B; data not shown). In vivo pulse labelling with bromodeoxyuridine (BrdU) revealed a similar level of BrdU-positive epithelial cells between the *Nbn*$^{CNS\text{-}del}$ and control mice at E13.5 and E15.5 (Fig. 2E, data not shown), but a significant decrease in BrdU-positive epithelial cells in the *Nbn*$^{CNS\text{-}del}$ lenses at E18.5 (Fig. 2D and E) compared to the control mice (Fig. 2C and E). These results indicate that *Nbn* deficiency causes proliferation defects of the lens epithelium, probably starting at the perinatal stage. As activation of cell cycle checkpoint protein p53 is responsible for Nbs1 deficiency-induced cerebellar developmental defects [20], we also evaluated whether p53 plays a role in Nbs1 deficiency-mediated microphthalmia and cataracts. Out of 86 *Nbn*$^{CNS\text{-}del}$;*Trp*53−/− mice monitored, the eyeballs and the lenses of mice were significantly smaller than those of control mice (Fig. 2F–H), and these mice progressively displayed cataracts after 3 weeks, similar to the *Nbn*$^{CNS\text{-}del}$ lenses. Thus, it is likely that development of microphthalmia and cataracts of the *Nbn*$^{CNS\text{-}del}$ lenses are independent of the p53 pathway.

### 3.3. *Architecture alterations in Nbn-deficient lens*

Histological analysis of the lenses from 3-week-old *Nbn*$^{CNS\text{-}del}$ mice revealed that instead of a monolayer of epithelial cells covering the anterior surface of the lens in the control animals (Fig. 3A), Nbs1-deficient lenses displayed less compacted epithelial cells (Fig. 3B). In addition, Nbs1-deficient lenses showed major architectural disorganization in the posterior subcapsular nucleated fibre cells, including irregular shape, pleiomorphic nuclei, occasional vaculation in the cytoplasm and the presence of ectopic nuclei in the organelle-free zone (OFZ) (Fig. 3B). DAPI staining further revealed these ectopic nuclei, with diffuse nuclear fragments or micronuclei from the subcapsular region to the OFZ in Nbs1-deficient lenses (Fig. 3D). These changes are accompanied by an alteration of the M/R/N complex function as indicated by loss of Mre11 nuclear localization in the lens nucleated cells (Fig. 3F), suggesting that Nbs1 deficiency disrupted the synchronization of denucleation, a terminal differentiation process, of the lens fibre cells.

To investigate Nbs1 deficiency-induced architectural alterations of the lens in more detail, we analyzed the lenses of 3-week-old mice by transmission electron microscopy. In sharp contrast to the control lenses (Fig. 3G, I and K), Nbs1-deficient lens epithelial cells frequently displayed cytoplasmic vaculation and abnormally shaped nuclei (Fig. 3H). In addition, Nbs1-deficient fibres showed diffuse electron-dense bodies that could represent condensed or fragmented nuclei (Fig. 3J), which most likely corresponded to the ectopic nuclei, fragmentation or micronuclei seen by light microscopy (Fig. 3B and D). Moreover, Nbs1-deficient fibre cells in the OFZ contained heterogeneous protein particles and large protein aggregates (Fig. 3L).

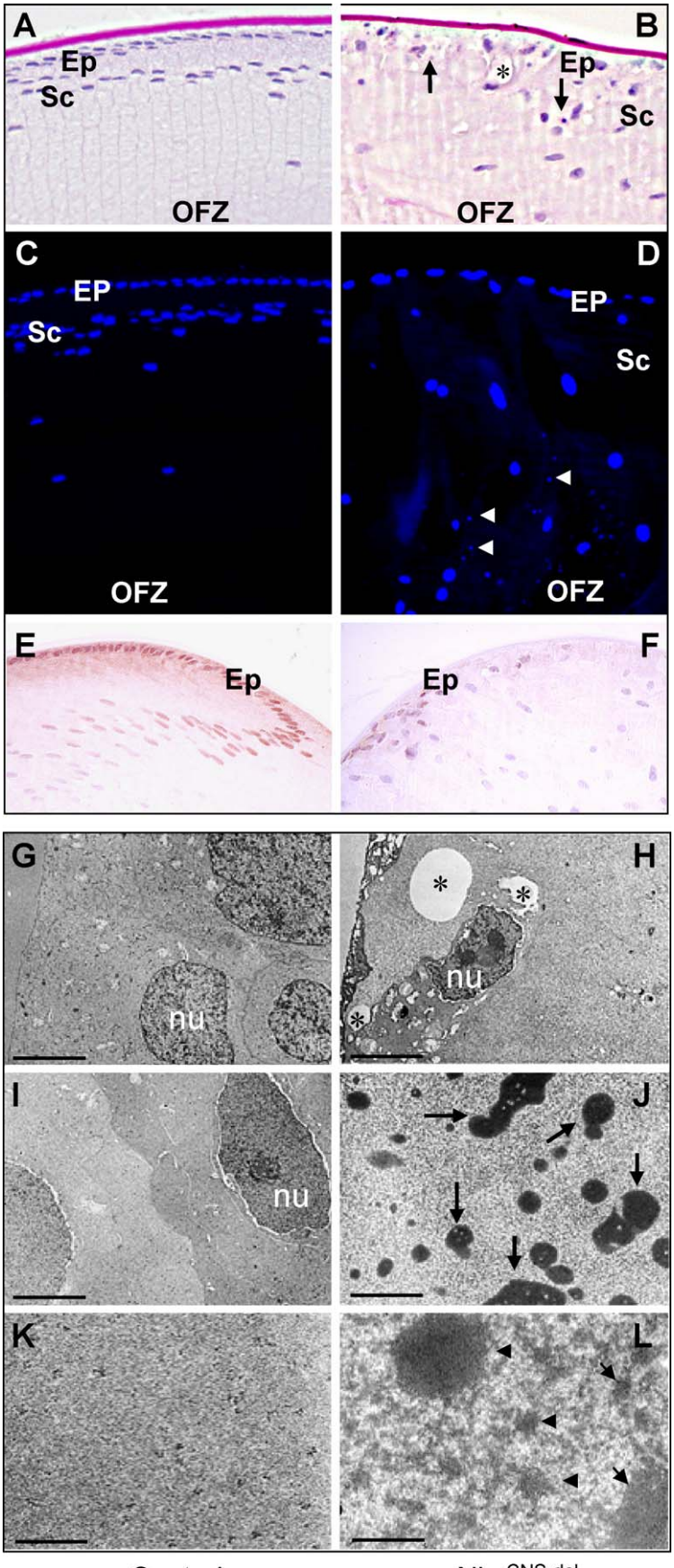
### 3.4. *Alteration of crystallin gene in Nbn-deficient lens*

Crystallins are major structural proteins of the lens encoded by α-, β- and γ-crystallin genes. Even a slight change in crystallin constituents and precipitation of the proteins, which may result from an imbalance in protein expression or mutation in the corresponding genes, can cause cataracts in humans [14,17,18]. To study whether the protein aggregates seen by electron microscopy were associated with alteration of the crystallins, we pooled lens RNA from 8 to 10 Nbs1-deficient and control mice, and examined the α-, β- and γ-crystallin transcripts by semi-quantitative RT-PCR (Fig. 4A). We found that expression of αA-, αB-, βA1/A3- and βB1-crystallin, as well as all γ-crystallins, in Nbs1-deficient lenses was dramatically reduced to 20–60% of control levels (Fig. 4A and B). To further explore whether Nbs1 deficiency-mediated genomic instability [20,27,28] could contribute to the dysregulation of crystallins, we analysed the crystallin loci and possible mutations in these genes. Southern-blot analysis revealed no apparent alteration in crystallin genomic loci in Nbs1-deficient lens (data not shown). We further sequenced RT-PCR products of α-, β-, γ-crystallin mRNA, and found no mutations in the crystallin coding sequences in Nbs1-deficient lenses (data not shown).

## 4. Discussion

In the present study, we have found that DDR molecules, the Mre11/Rad50/Nbs1 complex, are expressed in the mouse lenses, and depletion of *Nbn* did not alter the expression of *Mre11* and *Rad50* in the lenses. Interestingly, deletion of *Nbn* in the lens epithelial cells disrupted Mre11 nuclear localization in the lens nucleated cells, caused perinatal proliferation defects, and resulted in small lenses and microphthalmia in mice. These defects are independent of the p53 pathway, suggesting an involvement of additional targets in Nbs1 deficiency-mediated proliferation defects of the lens epithelial cells. In addition, we have shown a novel function of Nbs1 in terminal differentiation of the lens fibre cells and in the maintenance of lens transparency characterized by early onset of cataracts in Nbs1-deficient mice.

We have provided compelling evidence that Nbs1 deficiency causes disorganization of the epithelium and degenerative changes in the lens epithelial cells, indicating that cataract may be linked to defects in DDR and in processing DNA breaks in the lens epithelium. It has been proposed that proficient DNA repair is important for human lens epithelial cells to pre-


A
Ep
Sc
OFZ
B
Ep
Sc
OFZ
C
EP
Sc
OFZ
D
EP
Sc
OFZ
E
Ep
F
Ep
G
nu
H
nu
I
nu
J
K
L
Control
Nbn CNS-del

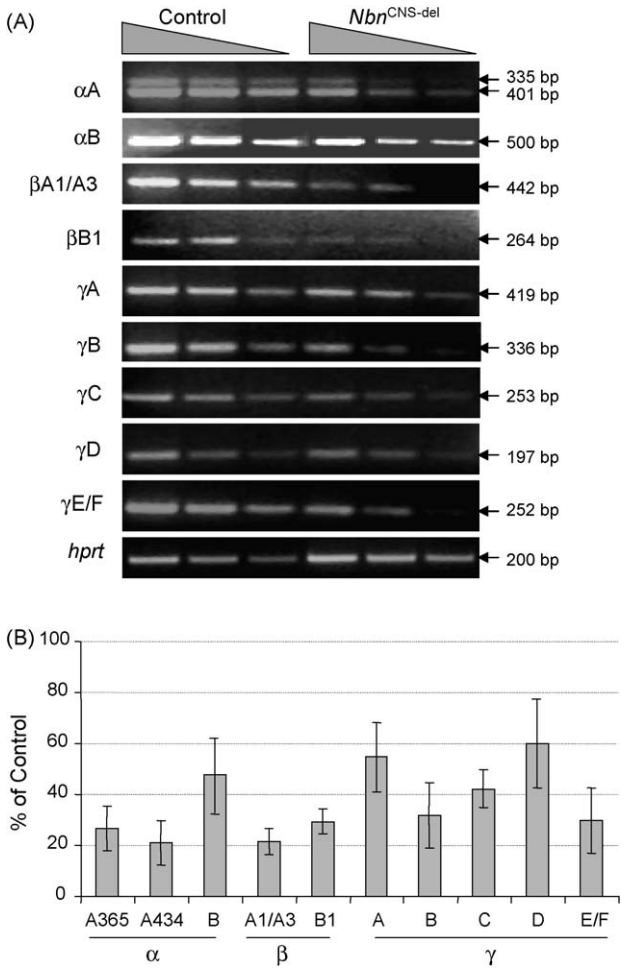



**Fig. 4 – Dysregulation of crystallin gene expression in Nbs1-deficient lenses. (A) Representative RT-PCR analysis of the α-, β- and γ-crystallin mRNA levels in the lenses of *Nbn*$^{CNS\text{-}del}$ and control mice. *Hprt* was used as internal control. cDNA template gradients were 1:2:5. (B) Histogram represents relative crystallin mRNA levels in *Nbn*-deficient lenses compared to that of controls. Two independent experiments for crystallin mRNA analysis were performed.**

vent UV- and oxidative stress-induced cataracts [29], and accumulation of DNA strand breaks has been implicated in human cataractous lesions [30,31]. Patients with chromosomal instability syndromes, such as ataxia-microcephaly-cataract syndrome, Fanconi anemia, and Cockayne syndrome frequently develop cataracts [8–12], yet the mechanism underlying these phenotypes remains unknown. Recently, *ATM* heterozygote mice were found to be susceptible to cataract induced by radiation, likely due to defects in DSB-mediated cell cycle checkpoints, DNA repair and apoptotic pathways in the lens epithelial cells [29]. In addition, over-expression of DNA polymerase β in the mouse lens epithelium has been found to result in early onset of severe cortical cataract, possibly due to increased mutation and genomic instability [32].

We have found that Nbs1 deficiency impairs fibre cell differentiation characterized by alterations of the lens fibre cell architecture and denucleation process. Although the presences of ectopic and fragmented nuclei during terminal differentiation represent a typical sign of DNA repair defects in Nbs1-deficient lens fibre cells, the development of microphthalmia and cataracts is independent of p53 signalling. It is possible that during chromatin degradation in the lens fibre cells, Nbs1 has an alterative role to increase DNA accessibility to endonucleases. Thus, the lens denucleation, an aspect of terminal differentiation, may be biochemically distinct from DNA repair and apoptosis. It is possible that reduction of overall nuclease activity in Nbs1-deficient lens may contribute to impaired denucleation (Supplementary Fig. S1). In support of this hypothesis, it has been shown that nuclease activity is essential for denucleation of the lens fibre cells during terminal differentiation, and deficiency in DNase II-like acid DNase (DLAD), the only known acid DNase required for lens fibre cell denucleation, causes incomplete DNA degradation in the lens fibre cells leading to cataract with long latency [19].

We have demonstrated that Nbs1 deficiency causes protein aggregation associated with dysregulated transcription of various crystallins. The mechanism by which Nbs1 regulates crystallin gene transcriptions is unknown, and whether the defects in gene expression are the cause, or the result, of the cataracts is presently unclear. While Nbs1 is required for activation of ATM in response to DSBs [33], and given the cumulative evidence indicating that ATM controls the activity of a number of transcription factors, such as AP-1 [34], its involvement in gene transcriptional regulation may be postulated. This hypothesis is further strengthened by the finding that the M/R/N complex has been shown to colocalize with the transcriptional histone acetyl transferase (HAT) cofactor TRRAP [35]. Finally, we cannot rule out that down-regulation of crystallin gene expression is a consequence of impaired differentiation of fibre cells that have not reached the full differentiation stage.

**Fig. 3 – Architecture alterations of Nbs1-deficient lenses. (A) H&E staining of the lenses from 3-week-old control mice reveals a surface monolayer of epithelial cells (Ep), elongating nucleated fibre cells at the subcapsule (Sc) of the lens and organelle-free zone (OFZ). (B) A representative *Nbn*$^{CNS\text{-}del}$ lens shows disruption of the epithelial cell layer, disorganization of subcapsule nucleated fibre cells with irregular shape of nuclei (arrows), and vacuoles (*) in the subcapsular fibres. DAPI staining of the control (C) and *Nbn*$^{CNS\text{-}del}$ lens sections (D) reveals ectopic and fragmented nuclei (arrow heads) scattered in the lens subcapsule as well as OFZ. Immunostaining of Mre11 showing its nuclear localization in control epithelial and fibre cells (E), and cytoplasmic localization in *Nbn*$^{CNS\text{-}del}$ lens nucleated cells (F). Original magnification: ×40. (G–L) Transmission electron microscopic analysis of control and *Nbn*-deficient lenses. The control lens shows normal subcellular structure of epithelial cells (G), nucleated (nu) fibre cells in the subcapsule (I) and cells in the OFZ with homogenized protein particles (K). *Nbn*-deficient lenses contain nuclei with irregular shape and vacuoles (*) in the epithelial cells (H), electron-dense bodies (arrows) in the subcapsular fibre cells (J), and less packed protein particles with aggregations (arrow heads) in the OFZ (L). Bars in G–J are 5 μm, in K and L are 1.5 μm.**

The paradox, however, is that cataracts have not been reported in patients with NBS and Mre11 hypomorphic A-T-like disorder [1,3]. This may be explained by the presence of truncated C-terminal Nbs1 proteins or hypomorphic mutations of *Mre11* in these patients [36,37], which may sustain partial function of the M/R/N complex. Investigating these processes using conventional inactivation of *Mre11*, *Rad50*, and *Nbn* in mice has been hampered by early embryonic lethality caused by null mutations [38–41] or truncated Nbs1, Mre11 protein expression [42–44]. Thus, cataracts in *Nbn* null eyes suggest that the hypomorphic mutations of *Nbn* may fail to reveal some of the essential functions of Nbs1 in vivo.

In conclusion, the present study using a "conditional approach" to delete *Nbn* demonstrates a role of Nbs1 in proliferation and terminal differentiation of the lens epithelial cells into fibres, perhaps independent of its role in the DDR complex. Thus, $Nbn^{CNS\text{-}del}$ mice provide a valuable tool to investigate further the role of Nbs1 in early lens development, and to dissect the molecular mechanisms of congenital cataracts that are associated with chromosomal instability syndromes, and age-related cataracts.

## Acknowledgements

We thank D. Galendo for her excellent work in the maintenance of the animal colonies and J. Michelon, A. Saidi, C. Piccoli, N. Lyandrat and C. Carreira for technical support. We are also grateful to Drs. J. Hall, J. Loizou, D.E. Barnes and J. Cheney for their critical reading and comments on the manuscript. Further thanks are due to anonymous Reviewers for their useful and constructive comments. This work was partially supported by the Comite de la Loire and the Comite de la Drôme de la Ligue Nationale contre le Cancer, France.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.dnarep.2006.05.004.

## REFERENCES

[1] M. Digweed, K. Sperling, Nijmegen breakage syndrome: clinical manifestation of defective response to DNA double-strand breaks, DNA Repair 3 (2004) 1207–1217.
[2] D. D'Amours, S.P. Jackson, The Mre11 complex: at the crossroads of DNA repair and checkpoint signalling, Nat. Rev. Mol. Cell Biol. 3 (2002) 317–327.
[3] T.H. Stracker, J.W. Theunissen, M. Morales, J.H. Petrini, The Mre11 complex and the metabolism of chromosome breaks: the importance of communicating and holding things together, DNA Repair 3 (2004) 845–854.
[4] Y. Shiloh, ATM and related protein kinases: safeguarding genome integrity, Nat. Rev. Cancer 3 (2003) 155–168.
[5] T. Uziel, Y. Lerenthal, L. Moyal, Y. Andegeko, L. Mittelman, Y. Shiloh, Requirement of the MRN complex for ATM activation by DNA damage, EMBO J. 22 (2003) 5612–5621.
[6] T. Stiff, C. Reis, G.K. Alderton, L. Woodbine, M. O'Driscoll, P.A. Jeggo, Nbs1 is required for ATR-dependent phosphorylation events, EMBO J. 24 (2005) 199–208.
[7] J.H. Lee, T.T. Paull, ATM activation by DNA double-strand breaks through the Mre11-Rad50-Nbs1 complex, Science 308 (2005) 551–554.
[8] Y. Ziv, M. Frydman, E. Lange, N. Zelnik, G. Rotman, C. Julier, N.G. Jaspers, Y. Dagan, D. Abeliovicz, H. Dar, Z. Borochowitz, M. Lathrop, R.A. Gatti, Y. Shiloh, Ataxia-telangiectasia: linkage analysis in a highly inbred Arab and Druze families and differentiation from ataxia-microcephaly-cataract syndrome, Hum. Genet. 88 (1992) 619–626.
[9] M. Weyl Ben Arush, J. Rosenthal, J. Dale, Y. Horovitch, G. Herzl, J. Ben Arie, Y. Ziv, Y. Shiloh, Ataxia telangiectasia and lymphoma: an indication for individualized chemotherapy dosing–report of treatment in a highly inbred Arab family, Pediatr. Hematol. Oncol. 12 (1995) 163–169.
[10] M. Merriman, J. Mora, J. McGaughran, Fanconi anemia and primary cataracts: first case, Ophthalmic Genet. 23 (2002) 253–255.
[11] A.A.H. Mahmoud, G.M. Yousef, I. Al-Hifzi, E.S. Diamandis, Cockayne syndrome in three sisters with varying clinicl presentation, Am. J. Med. Genet. 111 (2002) 81–85.
[12] P. Mohaghegh, I.D. Hickson, Premature aging in RecQ helicase-deficient human syndromes, Int. J. Biochem. Cell Biol. 34 (2002) 1496–1501.
[13] P.J. Francis, V. Berry, S.S. Bhattacharya, A.T. Moore, The genetics of childhood cataract, J. Med. Genet. 37 (2000) 481–488.
[14] J.F. Hejtmancik, M. Kantorow, Molecular genetics of age-related cataract, Exp. Eye Res. 79 (2004) 3–9.
[15] P.A. Asbell, I. Dualan, J. Mindel, D. Brocks, M. Ahmad, S. Epstein, Age-related cataract, Lancet 365 (2005) 599–609.
[16] R.L. Chow, R.A. Lang, Early eye development in vertebrates, Annu. Rev. Cell. Dev. Biol. 17 (2001) 255–296.
[17] P.J. Francis, V. Berry, A.T. Moore, S. Bhattacharya, Lens biology: development and human cataractogenesis, Trends Genet. 15 (1999) 191–196.
[18] J. Graw, The genetic and molecular basis of congenital eye defects, Nat. Rev. Genet. 4 (2003) 876–888.
[19] S. Nishimoto, K. Kawane, R. Watanabe-Fukunaga, H. Fukuyama, Y. Ohsawa, Y. Uchlyama, N. Hashida, N. Ohguro, Y. Tano, T. Morimoto, Y. Fukuda, S. Nagata, Nuclear cataract caused by a lack of DNA degradation in the mouse eye lens, Nature 424 (2003) 1071–1074.
[20] P.O. Frappart, W.M. Tong, I. Demuth, I. Radovanovic, Z. Herceg, A. Aguzzi, M. Digweed, Z.-Q. Wang, An essential function for Nijmegen Breakage Syndrome gene (NBS1) and DNA damage response in the prevention of ataxia and cerebellar defects, Nat. Med. 11 (2005) 538–544.
[21] J. Yang, W. Bian, X. Gao, L. Chen, N. Jing, Nestin expression during mouse eye and lens development, Mech. Dev. 94 (2000) 287–291.
[22] L.A. Donehower, M. Harvey, B.L. Slagle, M.J. McArthur, C.A. Montgomery Jr., J.S. Butel, A. Bradley, Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours, Nature 356 (1992) 215–221.
[23] W.M. Tong, M.P. Hande, P.M. Lansdorp, Z.Q. Wang, DNA strand break-sensing molecule poly(ADP-Ribose) polymerase cooperates with p53 in telomere function, chromosome stability, and tumor suppression, Mol. Cell Biol. 21 (2001) 4046–4054.
[24] S. Nishiguchi, H. Wood, H. Kondoh, R. Lovell-Badge, V. Episkopou, Sox1 directly regulates the gamma-crystallin genes and is essential for lens development in mice, Genes Dev. 12 (1998) 776–781.
[25] B.Z. Ring, S.P. Cordes, P.A. Overbeek, G.S. Barsh, Regulation of mouse lens fiber cell development and differentiation by the Maf gene, Development 127 (2000) 307–317.

[26] A. Desai-Mehta, K.M. Cerosaletti, P. Concannon, Distinct functional domains of nibrin mediate Mre11 binding, focus formation, and nuclear localization, Mol. Cell. Biol. 21 (2001) 2184–2191.
[27] I. Demuth, P.O. Frappart, G. Hildebrand, A. Melchers, S. Lobitz, L. Stockl, R. Varon, Z. Herceg, K. Sperling, Z.Q. Wang, M. Digweed, An inducible null mutant murine model of Nijmegen breakage syndrome proves the essential function of NBS1 in chromosomal stability and cell viability, Hum. Mol. Genet. 13 (2004) 2385–2397.
[28] B. Reina-San-Martin, M.C. Nussenzweig, A. Nussenzweig, S. Difilippantonio, Genomic instability, endoreduplication, and diminished Ig class-switch recombination in B cells lacking Nbs1, Proc. Natl. Acad. Sci. USA 102 (2005) 1590–1595.
[29] B.V. Worgul, L. Smilenov, D.L. Brenner, A. Junk, W. Zhou, E.J. Hall, Atm heterozygous mice are more sensitive to radiation induced cataracts than are their wild-type counterparts, Proc. Natl. Acad. Sci. USA 99 (2002) 9836–9839.
[30] B.V. Worgul, J. David, S. Odrich, G.R. Merriam Jr., C. Medvedovsky, J.C. Merriam, S.L. Trokel, C.R. Geard, Evidence of genotoxic damage in human cataractous lenses, Mutagenesis 6 (1991) 495–499.
[31] N.J. Kleimen, A. Spector, DNA single strand breaks in human lens epithelial cells from patients with cataract, Curr. Eye Res. 12 (1993) 423–431.
[32] R.W. Sobol, J.F. Foley, A. Nyska, M.G. Davidson, S.H. Wilson, Regulated over-expression of DNA polymerase β mediates early onset cataract in mice, DNA Repair 2 (2003) 609–622.
[33] E.S. Stavridi, T.D. Halazonetis, Nbs1 moving up in the world, Nat. Cell Biol. 7 (2005) 648–650.
[34] N. Weizman, Y. Shiloh, A. Barzilai, Contribution of the Atm protein to maintaining cellular homeostasis evidenced by continuous activation of the AP-1 pathway in Atm-deficient brains, J. Biol. Chem. 278 (2003) 6741–6747.
[35] F. Robert, S. Hardy, Z. Nagy, C. Baldeyron, R. Murr, U. Dery, J.Y. Masson, D. Papadopoulo, Z. Herceg, L. Tora, The transcriptional histone acetyltransferase cofactor TRRAP associates with the MRN repair complex and plays a role in DNA double-strand break repair, Mol. Cell Biol. 26 (2006) 402–412.
[36] G.S. Stewart, R.S. Maser, T. Stankovic, D.A. Bressan, M.I. Kaplan, N.G. Jaspers, A. Raams, P.J. Byrd, J.H. Petrini, A.M. Taylor, The DNA double-strand break repair gene hMRE11 is mutated in individuals with an ataxia-telangiectasia-like disorder, Cell 99 (1999) 577–587.
[37] R.S. Maser, R. Zinkel, J.H. Petrini, An alternative mode of translation permits production of a variant NBS1 protein from the common Nijmegen breakage syndrome allele, Nat. Genet. 27 (2001) 417–421.
[38] Y. Xiao, D.T. Weaver, Conditional gene targeted deletion by Cre recombinase demonstrates the requirement for the double-strand break repair Mre11 protein in murine embryonic stem cells, Nucleic Acids Res. 25 (1997) 2985–2991.
[39] G. Luo, M.S. Yao, C.F. Bender, M. Mills, A.R. Bladl, A. Bradley, J.H. Petrini, Disruption of mRad50 causes embryonic stem cell lethality, abnormal embryonic development, and sensitivity to ionizing radiation, Proc. Natl. Acad. Sci. USA 96 (1999) 7376–7381.
[40] J. Zhu, S. Petersen, L. Tessarollo, A. Nussenzweig, Targeted disruption of the Nijmegen breakage syndrome gene NBS1 leads to early embryonic lethality in mice, Curr. Biol. 11 (2001) 105–109.
[41] V. Dumon-Jones, P.O. Frappart, W.M. Tong, G. Sajithlal, W. Hulla, G. Schmid, Z. Herceg, M. Digweed, Z.-Q. Wang, Nbn heterozygosity renders mice susceptible to tumor formation and ionizing radiation-induced tumorigenesis, Cancer Res. 63 (2003) 7263–7269.
[42] B.R. Williams, O.K. Mirzoeva, W.F. Morgan, J. Lin, W. Dunnick, J.H. Petrini, A murine model of Nijmegen breakage syndrome, Curr. Biol. 12 (2002) 648–653.
[43] J. Kang, R.T. Bronson, Y. Xu, Targeted disruption of NBS1 reveals its roles in mouse development and DNA repair, EMBO J. 21 (2002) 1447–1455.
[44] J.W. Theunissen, M.I. Kaplan, P.A. Hunt, B.R. Williams, D.O. Ferguson, F.W. Alt, J.H. Petrini, Checkpoint failure and chromosomal instability without lymphomagenesis in Mre11 (ATLD1/ATLD1) mice, Mol. Cell 12 (2003) 1511–1523.