The c-myc proto-oncogene encodes a ubiquitously transcribed factor involved in the control of cell growth and implicated in inducing tumorogenesis. Understanding the function of c-Myc and its role in cancer depends upon the identification of c-Myc target genes. Nijmegen breakage syndrome (NBS) is a chromosomal-instability syndrome associated with cancer predisposition, radiosensitivity, and chromosomal instability. The NBS gene product, NBS1 (p95 or nibrin), is a part of the hMre11 complex, a central player associated with double-strand break (DSB) repair. NBS1 contains domains characteristic for proteins involved in DNA repair, recombination, and replication. Here we show that c-Myc directly activates NBS1. c-Myc-mediated induction of NBS1 gene transcription occurs in different tissues, is independent of cell proliferation, and is mediated by a c-Myc binding site in the intron 1 region of NBS1 gene. Overexpression of NBS1 in Rat1a cells increased cell proliferation. These results indicate that NBS1 is a direct transcriptional target of c-Myc and links the function of c-Myc to the regulation of DNA DSB repair pathway operating during DNA replication.

The c-myc proto-oncogene codes for a nuclear phosphoprotein ubiquitously expressed in somatic cells (1, 2). Alterations of the c-myc locus, caused by chromosomal translocation, amplification, retroviral insertion, or retroviral transduction, deregulate c-Myc expression and contribute to tumorogenesis in different species (1, 3–5). The c-Myc protein contains a carboxyl-terminal basic, helix-loop-helix and leucine-zipper domain, which associates with another basic, helix-loop-helix and leucine-zipper protein (MAX) as heterodimers, and an amino-terminal domain necessary for transcriptional transactivation (2, 6, 7). The heterodimeric complexes c-Myc-MAX are capable of binding DNA at a specific site (E-box) and activate transcription of downstream target genes by recruiting protein complexes that can regulate histone acetylation and modify chromatin structure (8–10).

The precise function of the c-Myc protein, and in particular the mechanism by which it promotes cell proliferation in normal and neoplastic cells, is not known. Using a variety of approaches, including genome-wide gene expression profiling, various c-Myc target genes have been identified and have been shown to be involved in heterogeneous functions, including cell cycle control, DNA synthesis, iron metabolism, protein synthesis, apoptosis, cell adhesion, and telomere maintenance (11). In Drosophila as well as in mammalian cells, c-Myc has been shown to promote cell growth (12, 13), although it has been recently reported that c-Myc may control organ and body size by regulating cell cycle entrance and cell number rather than cell size (14). A better understanding of the precise role of c-Myc depends upon the identification of the genes that are directly targeted by its transcriptional regulation.

Nijmegen breakage syndrome (NBS) is an autosomal recessive hereditary disorder characterized by microcephaly, a “bird-like” facial appearance, growth retardation, immunodeficiency, radiosensitivity, chromosomal instability, and predisposition to tumor formation (15, 16). The gene defective in NBS has been cloned, and the gene product, NBS1 (p95, nibrin) is a member of the DNA double-strand break (DSB) repair complex (hMre11 complex), including hMre11, hRad50, and NBS1 (15). Increased radiation sensitivity and radioreistant DNA synthesis of NBS fibroblasts are similar to the cellular features of ataxia telangiectasis cells (17), which is demonstrated by the recent results showing that ataxia telangiectasis-mutated protein phosphorylates NBS1 (18–20), linking these two proteins in the same pathway. NBS1 is a putative tumor suppressor gene as shown by the existence of NBS patients and some mutations discovered in different tumors (15, 21). However, NBS1 is expressed in highly proliferating tissues developmentally (22) and is located at sites of DNA synthesis through interaction with E2F (23). In addition, Mre11 complex is able to prevent DSB accumulation during chromosomal DNA synthesis to ensure cell cycle progression (24). Obviously, the roles of NBS1 are multiple, and some of them are still subject to intensive investigation.

Due to the correlation between c-Myc activity and physiological DNA synthesis during cell cycle progression, we investigated whether the expression of NBS1 could be regulated by c-Myc. In this report, we demonstrated that NBS1 is a direct c-Myc target gene, implicating that the DNA DSB repair pathway is regulated by c-Myc. The role of c-Myc during cell growth and proliferation is thereby linked to the physiological function of DNA DSB repair occurred during DNA replication to preserve the integrity of the genome and facilitate cell growth and proliferation.
Cell Lines and Plasmids—The lymphoblastoid cell lines, EREB.TC-Myc and EREB.MyecMAR60 cells were previously described (25–28). The NIH3T3 transfectable cell line was generated by transfecting pSV2 Neo (Invitrogen) and plasmid pMT2TMyc into NIH3T3 cells and selecting under G418 (250 μg/mL). The NIH3T3 transfectable cell line was generated by transfecting pSV2 Neo (Invitrogen) and plasmid pMT2TMyc into NIH3T3 cells and selecting under G418 (250 μg/mL). 293TMyc and HeLaMyc cell lines were generated by transfecting pSUPER Myc and pSV2Hygro into 293T cells or pSUPER Myc and pSV2 Neo into HeLa cells and selecting under hygromycin (100 μg/mL) or G418 (400 μg/mL). RatNBS cell lines were generated by transfecting the HeBOCMV plasmid into Rat1a cells and selecting using G418 (400 μg/mL). The pHeBOCMV plasmid was constructed by PCR-mediated generation of a 2.34-kb fragment of the full-length human NBS1 cDNA from the plasmid pBS-NBS1 obtained from Dr. P. Concannon (29). The primers used to generate this fragment are: 5′-CCGGTTTACCGCACCTGCGGCCC-3′ and 5′-TTGGCTCTAGAGGGCCGCGGCCC-3′. This fragment was subcloned into the Ndel-Nsil sites of HeBOCMV vector (25) to make the HeBOCMV plasmid expression vector, and it was verified by sequencing. The pSUPER Myc plasmid was generated by inserting the oligonucleotide of 5′-GATCCCCGATATGCAGTGGCAAGAGG-3′ and 5′-GATCGAGGCTCTCAGG-3′ upstream of the c-myc genomic region flanking the human NBS1 cDNA, which contains 396 bp 5′ upstream of the NBS1 transcription start site, first exon (88 bp), and 1 kb of intron 1. HindIII and BamHI sites were created to facilitate cloning. To construct the reporter construct driven by NBS1 promoter, the fragment mentioned above (containing the E-box) was subcloned into the fragment HindIII-BglII sites of the pXP2 vector to generate the NBSLuc construct (28). The ATG translation initiation codon of NBS1 was changed to GTG by site-directed mutagenesis to ensure translation of luciferase open reading frame and to make the NBSLuc1500 construct. This reporter construct was co-transfected with pMT2TMyc or its deletion mutants into 293T cells using the calcium phosphate transfection method, and their luciferase activities were assayed. The NBSLuc1500Mut construct was made by changing the sequence from CACCGTT to CACCCTG in the NBSLuc1500 construct by site-directed mutagenesis. 1 pmol of a c-myc wild type expression vector (pMT2TMyc) or a control vector (pMT2T) was co-transfected into 293T cells with 0.1 μg of reporter plasmids using the calcium phosphate transfection method. The total amount of transfected DNA and pMT2T sequences were kept constant in each experiment. A plasmid expressing the bacterial β-galactosidase gene (0.2 μg, pCMV-βgal) was also co-transfected in each experiment to serve as an internal control for transfection efficiency. At 48 h after transfection, cells were harvested and transcriptional activity was assayed as a function of luciferase activity. The values are expressed as luciferase activity after normalization with β-galactosidase activity for efficiency of transfection. Each transfection was performed in duplicate, and standard deviation values are shown. Data shown here are representative of three or more experiments from independent transfections.

Chromatin Immunoprecipitation Assay—A ChIP assay was performed following the detailed protocol as described previously (10, 32). Briefly, cells were cross-linked with 1% formaldehyde for 10 min and then the cross-linking was stopped by adding glycerol to a final concentration of 20% (v/v). Cells were washed twice with TBS (20 mM Tris, pH 7.5, 150 mM NaCl) and harvested in 5 ml of SDS buffer (50 mM Tris, pH 8.0, 0.5% SDS, 100 mM NaCl, 5 mM EDTA, and protease inhibitors). Cells were pelleted by centrifugation and suspended in 2 ml of IP buffer (100 mM Tris, pH 8.6, 0.3% SDS, 1.7% Triton X-100, 5 mM EDTA). Cells were sonicated with a 0.25-inch diameter probe for 15 s twice using an MSE Soniprep1500 sonicator (setting 18). For each immunoprecipitation, 1 ml of lysate was precleared by adding 50 μl of blocked protein A beads (50% protein A-Sepharose, Amersham Biosciences; 0.5 mg/ml bovine serum albumin, 0.2 mg/ml salmon sperm DNA) at 4°C for 1 h. Samples were spun, and the supernatants were incubated at 4 °C for 3 h with no antibody, or a monoclonal antibody either c-Bcl (SC-71, Santa Cruz Biotechnology) or c-Myc (SC-764, Santa Cruz Biotechnology). Immunocomplexes were recovered by adding 50 μl of blocked protein A beads and incubated overnight at 4 °C. Beads were successively washed with 1) mixed micelle buffer (20 mM Tris, pH 8.1, 150 mM NaCl, 5 mM EDTA, 5% w/v sucrose, 1% Triton X-100, 0.2% SDS), 2) buffer 500 (50 mM Hepes, pH 7.5, 0.1% w/v deoxycholic acid, 1% Triton X-100, 500 mM NaCl, 1 mM EDTA), 3) LiCl detergent wash buffer (10 mM Tris, pH 8.0, 0.5% deoxycholic acid, 0.5% Nonidet P-40, 250 mM LiCl, 1 mM EDTA), and 4) TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA) and then eluted with 1% SDS and 0.1 M NaHCO3. 20 μl of 5 μl of NaCl was added to the elutes, and the mixture was incubated at 65 °C for 4 h to reverse the cross-linking. After elution with proteinase K, the solution was phenol-chloroform extracted and ethanol-precipitated. DNA fragments were resuspended in 50 μl of 50 mM NaCl, 0.5 μl of the resuspended DNA was used in a PCR reaction for 25 cycles. 28 cycles were used for immunoprecipitated chromatin from EREB.TcMyc cells. The primers used were: 5′-ATTGGC- GCAAGATCTAATGTAGC-3′ and 5′-TGCGCATCCAGATGCGAAC- AATT-3′ for intron 1 fragment (440 bp in size), 5′-GTTGCTTGGAGT- AAGCCTTAAAAATCT-3′ and 5′-CTCTCATATGCTTGCAT-3′ for proximal promoter fragment (290 bp in size). Input was 2% of total input lysate. The data shown here are representative of two or more experiments from independent immunoprecipitations.

RESULTS

Activation of NBS1 by c-Myc in Different Physiological Conditions and in Multiple Cell Types—To characterize NBS1 as a potential c-Myc target gene, we used a human Epstein-Barr virus-immortalized B cells (CB33) engineered by transfection to express high levels of either c-Myc-MAX (CB-Max-Max) or MAX-MAX (CBMax) complexes, representing the two regulatory situations of high and low c-Myc function, respectively (25). The phenotypes of these cells were previously studied.
characterized and shown to be consistent with their differential c-Myc levels, because CBMMax cells display a short doubling time, have clonogenic properties in vitro and cause tumors in vivo, whereas CBMax cells proliferate slowly and lack any transformation-related phenotype (25). Northern blot analysis of these cell lines showed that the expression of NBS1 RNA was significantly increased (~2.5-fold) in CBMMax cells when compared with CB control or CBMax cells (Fig. 1A). To examine whether increased NBS1 expression was associated with increased protein levels, cell extracts from CB33 control, CBMyc.Max, and CBMax cells were analyzed by Western blot using an anti-NBS1 antibody and an anti-β-actin antibody as a control for protein loading.

**Fig. 1. Relationship between c-myc and NBS1 RNA and protein expression in lymphoblastoid cells (CB33).** A, direct correlation between c-myc and NBS1 gene expression. Northern blot analysis of poly(A) RNA from CB33 cells transfected with plasmid vectors expressing c-myc and MAX (CBMyc.Max), MAX (CBMax), or a control vector (CB control). The same blot was sequentially hybridized with the indicated probes (see “Experimental Procedures”). B, Western blot analysis of the cell lysates from the CB33 control, CBMyc.Max, and CBMax cells using an anti-NBS1 antibody and an anti-β-actin antibody as a control for protein loading.

**Fig. 2. Activation of NBS1 by c-Myc in EREB.TCmyc cells overexpressing c-Myc and NIH3T3 cells overexpressing c-Myc.** A, activation of NBS1 RNA expression by c-Myc in the absence of cell proliferation. Northern blot analysis of RNA from EREB.TCmyc cells upon estrogen (E2) withdrawal and c-myc activation by tetracycline (TC) withdrawal. The same blot was sequentially hybridized with an exon-3 genomic c-myc probe, an exon-1 c-myc probe, and a β-actin cDNA probe. B, activation of mouse Nbs1 RNA expression in NIH3T3 cells overexpressing c-Myc (NIH3T3/Myc) versus control NIH3T3 cells.

**Fig. 3. Regulation of NBS1 by endogenous c-Myc as demonstrated by RNAi experiments.** A, Western blot analysis showed the decrease in NBS1 levels in the 293T clone expressing small interfering RNA (293Tmcy) compared with the control 293T clone. The NBS1 protein level was decreased in the 293Tmcy clone. In addition, Fig. 3B showed that the endogenous c-Myc expression was repressed in three different HeLa clones expressing small interfering RNA (HeLaMyci clones) to repress endogenous c-myc versus the control HeLa clone. NBS1 is regulated by endogenous c-Myc, we performed RNA interference (RNAi) experiments on two different cell lines demonstrated the regulation of NBS1 by endogenous c-Myc.

**Activation of NBS1 by c-Myc in the Absence of de Novo Protein Synthesis—**To investigate whether c-Myc-mediated up-regulation of NBS1 gene expression was direct, we studied NBS1 expression in EREB (EREB.MycER TM) cells to repress endogenous c-myc versus the control 293Tmcy clone. By this approach and by simultaneous treatment with the protein synthesis inhibitor cycloheximide (CX), we examined whether NBS1 gene expression could be regulated upon activation of pre-existing c-MYCERTM in the absence of estradiol (E2) removal leads to growth arrest (G0/G1) associated with complete down-regulation of endogenous c-myc expression (Fig. 2A, +TC/−E2 lanes) (27). Because these cells have been transfected with a tetracycline (TC)-repressed c-myc vector, exogenous c-myc expression can then be induced by TC withdrawal. Induction of c-myc is not sufficient to cause cell cycle entrance, and the cells remain quiescent and viable for several days (26).

We analyzed NBS1 expression upon c-myc induction in the same cells by Northern blot analysis. The results showed that withdrawal of estrogen and tetracycline (causing induction of exogenous c-myc) causes a ~2.5-fold increase in the expression of NBS1 RNA (Fig. 2A, −TC/−E2 lane). Twenty-four hours after E2 withdrawal, cells were examined for proliferation by flow cytometry analysis of DNA content and found arrested at G0/G1 as previously described (26); no change in cell cycle activity was detectable after induction of c-myc by TC withdrawal. This result also indicates that c-Myc induces NBS1 expression in the absence of cell proliferation. The levels of mouse Nbs1 expression also increased (~3-fold) in NIH3T3 cells overexpressing c-Myc when compared with control NIH3T3 cells (Fig. 2B), indicating the activation of mouse Nbs1 by c-Myc in a fibroblast background. Taken together, these results indicate that increased c-myc expression is associated with increased NBS1 RNA and protein levels in different physiological conditions and in multiple cell types.

**Regulation of NBS1 by Endogenous c-Myc—**To test whether c-Myc induces NBS1 expression in the absence of cell proliferation, Northern blot analysis of RNA from EREB.TCmcy cells upon estrogen (E2) withdrawal and c-myc activation by tetracycline (TC) withdrawal. The same blot was sequentially hybridized with an exon-3 genomic c-myc probe, an exon-1 c-myc probe, and a β-actin cDNA probe. B, activation of mouse Nbs1 RNA expression in NIH3T3 cells overexpressing c-Myc (NIH3T3/Myc) versus control NIH3T3 cells.
de novo protein synthesis. Fig. 4A showed that TM treatment led to a significant (−6.5- to 8-fold) and rapid (detectable after 1 h) increase in NBS1 mRNA levels. This induction was only slightly abolished by co-treatment with CX (CX plus TM) (Fig. 4, B and C), indicating that induction of the NBS1 gene expression involves a mechanism that is mostly independent of de novo protein synthesis. Control experiments using EREB control cells treated with TM did not cause the induction of NBS1 expression (data not shown). The rapid kinetics of c-Myc-induced up-regulation and its independence of cellular proliferation and new protein synthesis are consistent with a direct effect of c-Myc activation on NBS1 gene expression.

**Activation of NBS1 by c-Myc Requires an E-box Site Located in the Intron 1 Region**—To ascertain whether c-Myc up-regulates NBS1 gene expression at the transcriptional level, we first investigated whether the NBS1 promoter region contained c-Myc-MAX binding sites. The genomic organization of the 5′ flanking region of human NBS1 gene, including exon 1, is schematically reported in Fig. 5A. This region includes 360 bp of upstream sequence from the NBS1 transcription start site, exon 1 (88 bp), and −1 kb of partial intron 1 sequence. An E-box site (CAGCTG) is located within intron 1. We analyzed whether the c-Myc-MAX binding site could mediate transcriptional activation of the NBS1 gene by c-Myc, by studying whether c-Myc could activate the transcription of a reporter gene linked to NBS1 promoter sequences. To this end, we constructed a reporter vector (NBSLuc1500, Fig. 5A) containing segments of the 5′-region, exon 1, and part of the intron 1 sequence of the NBS1 gene linked to the promoterless coding domain of the luciferase gene (Luc) (28). NBSLuc1500 was then co-transfected with either the control pMT2T or the pMT2TMyc expression vector into 293T cells. Fig. 5B showed that c-Myc was able to activate the expression of the NBSLuc1500 construct at levels (−2.5-fold) comparable to those observed for other c-Myc target genes in this type of assay. To determine whether the intronic E-box site was responsible for c-Myc-mediated activation, its sequence was changed from CAGCTG to CACCTG using site-directed mutagenesis, and the resulting reporter construct (NBSLuc1500mut) was tested for c-Myc responsiveness in transient co-transfection assay. The results (Fig. 5B) showed that this mutant construct could not be activated by c-Myc, thus indicating that the E-box within intron 1 of the NBS1 gene mediates transcriptional activation by c-Myc. To further characterize the mechanism involved in c-Myc-mediated transactivation of NBS1, we tested the function of c-Myc mutants lacking the transactivation domain (pMT2TMycΔ7–91) or heterodimerization domain (pMT2TMycΔ371–412) (35). These two mutants were unable to induce the expression of the NBSLuc1500 reporter gene (Fig. 5C), indicating that c-Myc-mediated activation of NBS1 transcription required both the transactivation domain and heterodimerization with MAX, as expected for physiological c-Myc function. To determine whether c-Myc binds to the intronic E box in vivo, a chromatin immunoprecipitation (ChIP) assay using CB33Myc.Max cells was performed (10, 32). Fig. 5D (panel a) showed that c-Myc, but not c-Rel, binds to the intronic E-box as demonstrated by the c-Myc immunoprecipitated chromatin containing the intronic fragment amplifiable to generate a 440-bp fragment. The control experiment carried out to amplify an NBS1 proximal promoter fragment (290 bp), which did not contain an E-box, failed to generate an amplifiable fragment (Fig. 5D, panel b). The 440-bp fragment was also amplifiable from the c-Myc-immunoprecipitated EREB.TCMyc(+TC/+E2) chromatin, demonstrating the binding of endogenous c-Myc to the intronic E-box site (Fig. 5D, panel c). In addition, the 440-bp fragment was not amplifiable in c-Myc-immunoprecipitated chromatin prepared from CBMax cells (Fig. 5D, panel d) or quiescent EREB.TCMyc(+TC/+E2) cells (data not shown). Positive control using the c-Myc-immunoprecipitated chromatin from CB-Myc.Max cells to amplify the promoter of a known c-Myc target gene, TERT (telomerase reverse transcriptase), generated an amplifiable fragment (data not shown) (32). Taken together, these results indicate that c-Myc can induce direct activation of the NBS1 transcription by binding to the E-box site within the NBS1 intron 1 region.

**Induction of Cell Proliferation in Rat1a Cells by Overexpression of NBS1**—To explore the role of NBS1 induction in c-Myc-induced cell growth and proliferation, we tested whether NBS1-overexpressing Rat1a cells exhibit accelerated growth and proliferation phenotypes. An NBS1 expression vector was stably transfected into Rat1a cells, and the transfected Rat1a clones (RatNBS) were tested for their ability to increase cell proliferation and DNA synthesis rate by MITT and BrdUrd incorporation assays. Fig. 6A showed that two different Rat1a clones (RatCMV) by immunoprecipitation and Western blot analysis. Increased cell proliferation rate (−70 to 100% increase) and DNA synthesis (−25% increase) were ob-
served in the RatNBS clones compared with the control RatCMV clones using MTT and BrdUrd incorporation assays (Fig. 6, B and C). These results indicate that overexpression of NBS1 in Rat1a cells increased cell proliferation and DNA synthesis rate, consistent with the role of NBS1 in c-Myc-mediated cell growth and proliferation.

DISCUSSION

These results present the first demonstration of a DNA DSB repair gene, NBS1, as a direct c-Myc target gene. Our results demonstrated that NBS1 is activated by c-Myc in different c-Myc-overexpressing cell lines. RNAi experiments showed the regulation of NBS1 by endogenous c-Myc. EREB.MycER experiments, transient transfection, and chromatin immunoprecipitation assays showed that the activation of NBS1 by c-Myc is direct. Nbs1 is expressed in highly proliferating tissues developmentally (22), coinciding with the timing of myc expression. Nbs1 null mice are embryonic lethal (36). Mutant blastocysts from these Nbs1 null mice showed greatly diminished expansion of the inner cell mass in culture, suggesting that Nbs1 mediates essential functions during cell growth and proliferation (36). Primary or immortalized fibroblasts from NBS patients contain hypomorphic mutants of NBS1 (37). These hypomorphic NBS cells grow slower than NBS cells comple-
mented with wild type NBS1 (38). In addition, mouse embryo fibroblast cells from Nbs1<sup>−−</sup> mice (similar to human mutants) have impaired cellular proliferation (39). The results of increased cellular proliferation in Rat1a cells overexpressing NBS1 (Fig. 6) are consistent with all of the above observations (22, 36, 38, 39). All these results support the role of increased NBS1 expression in promoting c-Myc-mediated cell growth and proliferation.

Although many c-Myc target genes were identified (1, 11, 40, 41), NBS1 represents one of the relatively limited numbers of genes that are direct as opposed to secondary targets for c-Myc transcripational activation. NBS1 represents the first c-Myc target gene linked to DNA DSB repair, recombination, and replication. Recent experiments using the serial assessment of gene expression approach also identified APEX/Ref-1, BRCA1, and MSH2 as putative c-Myc target genes related to DNA repair (41). However, these putative target genes were not yet extensively characterized. In Saccharomyces cerevisiae, DNA repair genes are required during DNA replication to preserve the integrity of the genome (42). Given the fact that spontaneous DNA DSBs are generated frequently during DNA replication, activation of NBS1 by c-Myc may be required physiologically to preserve the integrity of the genome and facilitate DNA synthesis/repllication during cell cycle progression in highly proliferating tissues. Prevention of DSB accumulation by Mre11 complex as demonstrated in Xenopus experiments is consistent with this model (24). Taken together, NBS1 is the first c-Myc target gene linked to DNA DSB repair, thereby further elucidating the molecular mechanism of cell growth promotion induced by c-Myc.

Acknowledgements—We thank Dr. R. Dalla-Favera for critical comments on the manuscript and Drs. P. Concannon and R. Agami for the generous gift of full-length human NBS1 cDNA and pSUPER plasmid. We thank C. T. Lee, C. P. Lin, S. F. Tseng, and Y. Y. Chen for their excellent technical assistance. K. J. W. thanks Dr. S. Y. Shieh of the Academia Sinica for the initial experiments performed at her laboratory.

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c-Myc Directly Regulates the Transcription of the NBS1 Gene Involved in DNA Double-strand Break Repair
Yu-Chi Chiang, Shu-Chun Teng, Yi-Ning Su, Fon-Jou Hsieh and Kou-Juey Wu

J. Biol. Chem. 2003, 278:19286-19291.
doi: 10.1074/jbc.M212043200 originally published online March 13, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M212043200

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Additions and Corrections

Vol. 278 (2003) 19286–19291

c-Myc directly regulates the transcription of the NBS1 gene involved in DNA double-strand break repair.

Yu-Chi Chiang, Shu-Chun Teng, Yi-Ning Su, Fon-Jou Hsieh, and Kou-Juey Wu

Page 19287: The last sentence in the first paragraph is incorrect. The corrected sentence should read:

“The pSUPERMyci plasmid was generated by inserting the oligo of 5'-GATCCCGATTCGAGCTGCTGCCCACCCTTCAAGAGAGGGTGCCAGCAGCTGAATTATTGGAAAA-3' into the pSUPER plasmid provided by Dr. R. Agami (30).”

Vol. 278 (2003) 52102–52115

Pleiotropic effects of Ubp6 loss on drug sensitivities and yeast prion are due to depletion of free ubiquitin pool.

Tatiana A. Chernova, Kim D. Allen, Lisa M. Wesoloski, John R. Shanks, Yury O. Chernoff, and Keith D. Wilkinson

Page 52109, Fig. 5A: The Ubp6 blot was flipped, and labeling in rows 4 and 5 was incorrect. The correct Fig. 5A is shown below:

![Fig. 5A](image_url)