DOUBLE-STRAND BREAKS IN CHROMOSOMAL DNA CAN ARISE DURING THE COURSE OF DNA REPLICATION, AND CAN ALSO BE INTRODUCED BY IONIZING RADIATION AND GENOTOXIC CHEMICALS. EFFECTIVE REPAIR OF DNA DOUBLE STRAND BREAKS (DSBs) IS ESSENTIAL FOR THE GENETIC INTEGRITY OF CELLS, AND CAN BE ADDRESSED BY AT LEAST TWO DISTINCT MECHANISMS: HOMOLOGOUS RECOMBINATION (HR) AND NON HOMOLOGOUS END-JOINING (NHEJ) (1). THE MRE11/RAD50 COMPLEX (M/R) IS CONSERVED IN ALL THREE BIOLOGICAL KINGDOMS AND HAS BEEN SHOWN TO PLAY IMPORTANT ROLES IN BOTH THE HR AND NHEJ PATHWAYS OF DSB REPAIR (2-5). MUCH OF THE EVIDENCE FOR THIS COMES FROM BUDDING YEAST, IN WHICH THE MRE11 AND RAD50 GENE PRODUCTS HAVE BEEN STUDIED EXTENSIVELY AND FOUND TO ALSO BE REQUIRED FOR MEIOTIC RECOMBINATION AND TELOMERE MAINTENANCE. M/R ASSOCIATES WITH A THIRD COMPONENT IN EUKARYOTIC CELLS (NBS1 IN MAMMALS, XR52 IN S. CEREVISIAE) THAT LINKS M/R TO DNA DAMAGE-INDUCED CELL CYCLE CHECKPOINTS (6-9).

The Nbs1 polypeptide has clinical significance because mutations in the NBS1 gene have been identified as causal factors in the human autosomal recessive genetic disorder Nijmegen Breakage Syndrome (NBS) (10). Patients with NBS exhibit radiation sensitivity, immune system deficiency, and a high rate of malignancy (11). At the cellular level, abnormalities include a defective S-phase checkpoint response and an elevated rate of chromosomal breakage and translocations, although few overt deficiencies in DNA repair. It was recently demonstrated that the most common NBS allele, 657del5, can generate a C-terminal polypeptide through the use of an internal ribosome entry site upstream of the 5' nucleotide deletion (12). This translation product (p70) is capable of binding Mre11 and thus the 657del5 NBS allele is very likely a hypomorphic allele that can supply a subset of the normal functions of Nbs1.


USING A RECOMBINANTbaculovirus SYSTEM, WE HAVE PREVIOUSLY EXPRESSED THE HUMAN MRE11, RAD50, AND NBS1 PROTEINS TOGETHER AND FOUND THAT THEY FORM A LARGE PROTEIN COMPLEX WHICH EXHIBITS SEVERAL DISTINCT ENZYMATIC ACTIVITIES ON DNA SUBSTRATES. THE MRE11 PROTEIN CONTAINS HIGHLY CONSERVED PHOSPHOESTERASE MOTIFS THAT ARE RESPONSIBLE FOR THE MANGANESE-DEPENDENT NUCLEASE ACTIVITIES OF M/R/N. BY ITSELF AND IN ASSOCIATION WITH RAD50 AND NBS1, MRE11 EXHIBITS A DISTRIBUTIVE, 3' TO 5' EXONUCLEASE ACTIVITY ON BLUNT AND 3' RECESSIVE ENDS (18,19), AS WELL AS A WEAK ENDONUCLEASE ACTIVITY ON DISTORTED DNA SUBSTRATES SUCH AS HAIRPIN STRUCTURES. ASSOCIATION OF NBS1 WITH MRE11 STIMULATES THE ENDONUCLEASE FUNCTION TO ACT ON HAIRPIN STRUCTURES AND ON 3' OVERHANGS (20), ALTHOUGH NBS1 ITSELF HAS NO APPARENT ENZYMATIC ACTIVITIES. M/R CAN ALSO CATALYZE A LIMITED DNA UNWINDING REACTION ON DNA ENDS THAT IS STIMULATED BY ATP AND REQUIRES NBS1 (20).

In this study, we have expressed and characterized mutant M/R/N complexes equivalent to those present in NBS and ATLD patients. We found that the truncated version of Nbs1 found in NBS cells (p70) associates with Mre11 and

Proofs and reprints to: University of Texas at Austin Molecular Genetics & Microbiology 1 University Station A5000 - Austin, TX 78712-0162 - USA - e-mail: tpaull@intron.icmb.utexas.edu
Rad50 to form a triple complex similar to the wild-type enzyme. This complex of Mre11, Rad50, and p70 (M/R/N(p70)) exhibits wild-type levels of Mre11 exo- and endo-nuclease activity, and also shows nucleotide-dependent DNA binding and DNA unwinding at levels similar to the normal complex. While the exact biochemical activities of M/R/N are still under investigation, our analysis shows that every enzymatic function we can measure in vitro is essentially unchanged in a M/R/N(p70) mutant compared to the wild-type complex. This observation is not unexpected, considering that NBS cells have few overt defects in DNA repair but primarily exhibit deficiencies in checkpoint-related DNA damage responses (11). Other studies have also demonstrated that the N-terminus of Nbs1 that includes the forkhead and BRCT domains is necessary and sufficient for focus formation at sites of DNA damage, but that the C-terminus is required for survival of cells following ionizing radiation treatment (21-23).

We have also expressed and characterized mutant complexes equivalent to those present in ATLD1/2 and ATLD3/4 patients. The R633STOP truncation allele of Mre11 found in ATLD1/2 patients associated with Rad50 and Nbs1 similar to wild-type Mre11, although association of Nbs1 with the N117S mutant (ATLD3/4) was significantly reduced and variable. Both of the ATLD mutant complexes exhibited normal levels of Mre11 exonuclease activity, but reduced levels of endonuclease activity compared to the wild-type enzyme. ATLD cells, like A-T cells, do not show an overt defect in DNA repair (17), although a partial loss in endonuclease function could result in repair deficiencies that would be difficult to assess using assays for whole-genome recovery.

Recent evidence from a number of laboratories has suggested that full-length Nbs1 is required for the phosphorylation of some substrates by the ATM protein kinase. In NBS cells, which express only the p70 version of Nbs1, ATM phosphorylation of p53, Chk2, and SMCl is reduced or absent (24-28). This deficiency in ATM activity is most apparent in response to low doses of radiation (1 to 5 Gray), and is only observed in vivo. ATM protein extracted from NBS cells is active in vitro (25), thus ruling out the possibility that the ATM protein in NBS cells is lacking an essential modification or co-factor. In some cases, the phosphorylation of Nbs1 on multiple serine residues by ATM has also been shown to be necessary for efficient phosphorylation of other substrates by ATM (24,28), although other studies have yielded conflicting evidence (26,27). At present it is clear that the M/R/N complex is not simply a substrate of ATM, but also plays a role in facilitating ATM activities on other substrates and is therefore upstream as well as downstream of ATM in the DNA damage signaling pathway. The biochemical basis of this cooperative relationship is currently not understood, and biophysical effects of Nbs1 phosphorylation are not known.

To begin to answer these questions, we are investigating the effects of M/R/N on ATM kinase activity. Preliminary evidence suggests that the M/R/N complex can stimulate ATM phosphorylation of some substrates in vitro, an observation that may provide a molecular explanation for the observed effects of M/R/N on ATM activity in vivo.

References